

# BMJ Open

BMJ Open is committed to open peer review. As part of this commitment we make the peer review history of every article we publish publicly available.

When an article is published we post the peer reviewers' comments and the authors' responses online. We also post the versions of the paper that were used during peer review. These are the versions that the peer review comments apply to.

The versions of the paper that follow are the versions that were submitted during the peer review process. They are not the versions of record or the final published versions. They should not be cited or distributed as the published version of this manuscript.

BMJ Open is an open access journal and the full, final, typeset and author-corrected version of record of the manuscript is available on our site with no access controls, subscription charges or pay-per-view fees (<http://bmjopen.bmj.com>).

If you have any questions on BMJ Open's open peer review process please email [info.bmjopen@bmj.com](mailto:info.bmjopen@bmj.com)

# BMJ Open

## Towards biomarkers for outcomes after pancreatic ductal adenocarcinoma and ischemic stroke, with focus on (co-)morbidity and aging / cellular senescence (SASKit): protocol for a prospective cohort study

|                               |   |
|-------------------------------|---|
| Journal:                      | <i>BMJ Open</i>   |
| Manuscript ID                 | bmjopen-2020-039560   |
| Article Type:                 | Protocol  |
| Date Submitted by the Author: | 21-Apr-2020   |
| Complete List of Authors:     | <p>Henze, Larissa; Rostock University Medical Center, Department of Hematology, Oncology and Palliative Medicine, Department of Medicine III, Research Focus Oncology, Rostock University Medical Center</p> <p>Walter, Uwe; Rostock University Medical Center, Department of Child and Adolescence Psychiatry and Neurology, Centre for Transdisciplinary Neurosciences Rostock, Rostock University Medical Center</p> <p>Murua Escobar, Hugo; Rostock University Medical Center, Department of Hematology, Oncology and Palliative Medicine, Department of Medicine III, Research Focus Oncology, Rostock University Medical Center</p> <p>Junghanß, Christian; Rostock University Medical Center, Department of Hematology, Oncology and Palliative Medicine, Department of Medicine III, Research Focus Oncology, Rostock University Medical Center</p> <p>Jaster, Robert; Rostock University Medical Center, Department of Gastroenterology, Research Focus Oncology, Rostock University Medical Center</p> <p>Köhling, Rüdiger; Rostock University Medical Center, Oscar Langendorff Institute of Physiology, Centre for Transdisciplinary Neurosciences Rostock, Rostock University Medical Center, Ageing of Individuals and Society, Interdisciplinary Faculty, Rostock University</p> <p>Lange, Falko; Rostock University Medical Center, Oscar Langendorff Institute of Physiology, Centre for Transdisciplinary Neurosciences Rostock, Rostock University Medical Center</p> <p>Salehzadeh-Yazdi, Ali; University of Rostock, Department of Systems Biology and Bioinformatics</p> <p>Wolkenhauer, Olaf; University of Rostock, Department of Systems Biology and Bioinformatics, Centre for Transdisciplinary Neurosciences Rostock, Rostock University Medical Center</p> <p>Hamed, Mohamed; Rostock University Medical Center, Institute for Biostatistics and Informatics in Medicine and Ageing Research, Research Focus Oncology, Rostock University Medical Center</p> <p>Barrantes, Israel; Rostock University Medical Center, Institute for Biostatistics and Informatics in Medicine and Ageing Research</p> <p>Palmer, Daniel; Rostock University Medical Center, Institute for Biostatistics and Informatics in Medicine and Ageing Research</p> <p>Möller, Steffen; Rostock University Medical Center, Institute for Biostatistics and Informatics in Medicine and Ageing Research</p> <p>Kowald, Axel; Rostock University Medical Center, Institute for Biostatistics and Informatics in Medicine and Ageing Research</p> |

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

|           |  |
|-----------|--|
|           | Heussen, Nicole; RWTH Aachen University, Department of Medical Statistics, Research Focus Oncology, Rostock University Medical Center<br>Fuellen, Georg; Rostock University Medical Center, Institute for Biostatistics and Informatics in Medicine and Ageing Research, Centre for Transdisciplinary Neurosciences Rostock, Rostock University Medical Center, Research Focus Oncology, Rostock University Medical Center, Ageing of Individuals and Society, Interdisciplinary Faculty, Rostock University |
| Keywords: | Health informatics < BIOTECHNOLOGY & BIOINFORMATICS, Immunology < NATURAL SCIENCE DISCIPLINES, Thromboembolism < CARDIOLOGY, Molecular aspects < ONCOLOGY, Stroke < NEUROLOGY  |
|           |  |





I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our [licence](#).

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which [Creative Commons](#) licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

## Towards biomarkers for outcomes after pancreatic ductal adenocarcinoma and ischemic stroke, with focus on (co-)morbidity and aging / cellular senescence (SASKit): protocol for a prospective cohort study

Larissa Henze\*<sup>1,##</sup>, Uwe Walter\*<sup>2,#</sup>, Hugo Murua Escobar<sup>1,##</sup>, Christian Junghanß<sup>1,##</sup>, Robert Jaster<sup>3,##</sup>, Rüdiger Köhling<sup>4,#,###</sup>, Falko Lange<sup>4,#</sup>, Ali Salehzadeh-Yazdi<sup>5</sup>, Olaf Wolkenhauer<sup>5,#</sup>, Mohamed Hamed<sup>6,##</sup>, Israel Barrantes<sup>6</sup>, Daniel Palmer<sup>6</sup>, Steffen Möller<sup>6</sup>, Axel Kowald<sup>6</sup>, Nicole Heussen\*\*<sup>7</sup>, Georg Fuellen\*\*<sup>6,#,##,###</sup>

\*joint first authors

\*\*joint corresponding authors: [nheussen@ukaachen.de](mailto:nheussen@ukaachen.de), [fuellen@uni-rostock.de](mailto:fuellen@uni-rostock.de)

1 Rostock University Medical Center, Department of Hematology, Oncology and Palliative Medicine, Department of Medicine III, Rostock, Germany

2 Rostock University Medical Center, Department of Neurology, Rostock, Germany

3 Rostock University Medical Center, Department of Gastroenterology, Rostock, Germany

4 Rostock University Medical Center, Oscar Langendorff Institute of Physiology, Rostock, Germany

5 University of Rostock, Department of Systems Biology and Bioinformatics, Rostock, Germany

6 Rostock University Medical Center, Institute for Biostatistics and Informatics in Medicine and Ageing Research, Rostock, Germany

7 RWTH Aachen, Department of Medical Statistics, Aachen, Germany

# Centre for Transdisciplinary Neurosciences Rostock, Rostock University Medical Center ## Research Focus Oncology, Rostock University Medical Center ### Ageing of Individuals and Society, Interdisciplinary Faculty, Rostock University

### Abstract

**Introduction:** Aging-related processes such as cellular senescence are believed to underlie the accumulation of diseases in time, causing (co-)morbidity, including cancer, thromboembolism and stroke. Intervening into these processes may delay, stop or reverse morbidity. To study the link between (co-)morbidity and aging, by exploring biomarkers and molecular mechanisms of disease-triggered deterioration, we will recruit 50 patients with pancreatic ductal adenocarcinoma, 50 patients with (thromboembolic) ischemic stroke and 50 controls, at Rostock University Medical Center.

**Methods and Analysis:** We will gather routine blood data, clinical performance measurements and patient-reported outcomes at up to 9 points in time, and in-depth transcriptomics & proteomics at two early time points. Aiming for clinically relevant biomarkers, the primary outcome is a composite of probable sarcopenia, clinical performance (described by ECOG Performance Status for patients with pancreatic ductal adenocarcinoma and the Modified Rankin Scale for patients with stroke) and quality of life. Further outcomes cover other aspects of morbidity such as cognitive decline, and of comorbidity such as vascular or cancerous events. The data analysis is comprehensive in that it includes biostatistics & machine learning, both following standard role models & additional explorative approaches. *Predictive* biomarkers for interventions addressing senescence may become available if the biomarkers that we find are predominantly related to aging / cellular senescence. Similarly, *diagnostic* biomarkers will be explored for their relationship to aging / cellular senescence. Our findings will require validation in independent studies, and our dataset shall be useful to validate the findings of other studies. In some of the explorative analyses, we shall include insights from systems biology modeling as well as insights from preclinical animal models. We humbly suggest that our detailed study protocol and data analysis plan may also guide other biomarker exploration trials. **Ethics and Dissemination:** The study was approved by the local ethics committee, registered at the German Clinical Trials Register, and results will be published following standard guidelines.

### Article summary

Strengths and limitations of this study:

- In-depth measurements of both relevant outcomes and potential biomarkers.
- Comparatively low number of participants, for both patients and controls.
- In-depth and detailed data analysis plan.
- Investigation of the deterioration of health and (co-)morbidity, not just of survival.
- Two co-morbid diseases investigated in almost identical ways in two sub-studies.

### Introduction

**Study Rationale and Aims.** The primary aim of the SASKit (“Senescence-Associated Systems diagnostics Kit for cancer and stroke”) study is to discover a set of molecular biomarkers for outcomes after pancreatic ductal adenocarcinoma (PDAC) and ischemic stroke (IS), which are specifically useful to predict disease-triggered deterioration of health (“disease deterioration” for short) in terms of probable sarcopenia<sup>1</sup>, reduced clinical performance and quality of life (QoL). The outcomes also include the (co-)morbidity of vascular events (here defined as stroke, myocardial infarction, and venous or arterial thromboembolism) in patients with PDAC, which are observed frequently apart from sarcopenia. Also included is the (co-)morbidity of any kind of cancer and of cognitive decline following IS. Moreover, we consider mortality, as the most canonical outcome. Following up on the primary aim, we will investigate the nature of the molecular biomarkers to find out whether cellular senescence and other aging-associated processes are contributing to disease deterioration. As a secondary aim, we will search for *diagnostic* biomarkers related to cellular senescence and other aging-related processes that may differentiate healthy controls from PDAC or IS patients. Therefore, in the following we motivate our study by describing the prevalence and the outcomes of PDAC and IS, the known predictors of these outcomes, and the specific prevalence of co-morbidity and known predictors for this co-morbidity. The role of cellular senescence in aging and disease is described in Box 1. The background of the cancerous and vascular comorbidity is described in Box 2. Avoiding unclear or circular terminology, we define a biomarker in a very general fashion, simply as a feature (data point)  $f_1$  that successfully predicts another feature  $f_2$  at a later time-point<sup>2</sup>, in a biomedical context. Here, features may be composite ones, based on the measurement of individual features. Often, feature  $f_1$  refers to molecular data, while feature  $f_2$  refers to phenotypic data, such as clinical outcomes. Ultimately, we aim to identify biomarkers that are easy to measure, and that are then validated in other studies to predict a clinically relevant outcome.

**Pancreatic ductal adenocarcinoma: prevalence and outcomes.** The incidence of pancreatic cancer is increasing; in 2017 the global incidence was 5.7 per 100,000 person-years<sup>3</sup>. Age is the most important risk factor, and incidence peaks at 65 to 69 years in males and 75 to 79 years in females<sup>3</sup>. Pancreatic ductal adenocarcinoma (PDAC) is the most common histological type of pancreatic cancer<sup>4</sup>. The disease is characterized by late clinical presentation<sup>5</sup>, early metastases and poor prognosis, with a one-year survival rate in Europe of only 15%<sup>6</sup>. Many patients have unresectable disease at the time of diagnosis, either as locally advanced disease or already with metastases. Therefore therapy is palliative consisting of chemotherapy and/or best supportive care. Disease deterioration with weight loss and low muscle strength, that is, cachexia and sarcopenia<sup>7</sup>, will follow, for some patients rapidly (within a few weeks) and for others during a longer interval of one or two years. Recent developments in oncology have not shown much benefit in clinical trials of patients with PDAC<sup>8</sup>. Inflammation, desmoplasia and early metastases are deemed responsible for the difficulties in targeting the disease. Moreover, vascular events are frequent problems in the course of PDAC and may contribute to disease deterioration or early death. Venous thromboembolism is the most common event occurring in up to 34% of patients with metastatic PDAC<sup>9,10</sup>, but arterial ischemic events, like stroke, are also reported

1  
2  
3 <sup>11-14</sup>, see also Box 2. Therefore, deterioration and mortality in PDAC can not only be explained by tumor  
4 progression as such, but other factors like sarcopenia/cachexia and vascular events contribute as well.  
5 Furthermore, we suggest that the underlying cause of all these factors are aging-related processes  
6 such as cellular senescence and chronic inflammation.  
7

8  
9 ***Pancreatic ductal adenocarcinoma: known biomarkers and clinical scores.*** In PDAC patients there is  
10 a lack of established scores describing the risk of disease deterioration and the risk of  
11 sarcopenia/cachexia in particular. Referring to the endpoint of overall survival, some recent studies  
12 tried to establish inflammation-based scores to better characterize outcome in PDAC. In a  
13 retrospective analysis of 386 patients with PDAC of different stages, CRP/Alb ratio, neutrophil–  
14 lymphocyte ratio (NLR), platelet–lymphocyte ratio (PLR) and modified Glasgow prognostic score  
15 (mGPS) were studied <sup>15</sup>. In patients with locally advanced and metastatic disease, the CRP/alb ratio  
16 was an independent factor of poor survival <sup>15</sup>. Another retrospective study evaluating CA19-9, CEA,  
17 CRP, LDH and bilirubin levels in locally advanced and metastatic pancreatic cancer patients treated  
18 with chemotherapy showed an independent prognostic significance for overall survival only for CA 19-  
19 9 decline during treatment <sup>16</sup>. Other studies have evaluated risk factors for thromboembolic events in  
20 pancreatic cancer patients and more generally in patients with cancer <sup>17</sup> (see also Box 2). The Khorana  
21 score, developed more than ten years ago, is widely used to estimate venous thromboembolic risk in  
22 the population of cancer patients <sup>18</sup>; it integrates standard laboratory parameters (platelet count,  
23 hemoglobin, leukocyte count), body mass index (BMI) and the cancer site (with pancreatic cancer and  
24 gastric cancer classified as very high risk). Still, its performance was questioned in a retrospective  
25 cohort of pancreatic cancer patients <sup>19</sup> and in a prospective cohort study of patients with different  
26 cancer types, among them 109 with pancreatic cancer <sup>17</sup>. The clinical association of PDAC,  
27 sarcopenia/cachexia and thromboembolism is well-described <sup>11</sup>, but still not understood in its  
28 pathophysiology <sup>20</sup>. Within the SASKit study we aim to identify biomarkers and molecular mechanisms  
29 contributing to this clinical association, by investigating their relation to clinically relevant outcomes.  
30  
31  
32  
33  
34  
35

36  
37 ***Ischemic stroke, prevalence and outcomes.*** Ischemic stroke (IS) occurs in the German population with  
38 an incidence of 236 per 100,000 per year <sup>21</sup>. The mean age of acute stroke patients is 73-74 years, with  
39 more than 80% of patients being over 60 years old. After a first stroke, nearly 5% of patients suffer a  
40 second stroke within a year. Mortality after IS is about 12% within one year and about 30% within five  
41 years <sup>21</sup>. Mild to moderately disabled stroke survivors showed an elevated prevalence of sarcopenia  
42 >6 months after onset of stroke compared with non-stroke individuals (13.2% vs 5.3%) <sup>22</sup>. The  
43 mechanisms underlying sarcopenia include loss of muscle mass, reduction of fibre cross-sectional area  
44 and increased intramuscular fat deposition occurring between 3 weeks and 6 months after stroke in  
45 both paretic and non-paretic limbs <sup>23</sup>. Comorbid, or subsequent cancer may facilitate sarcopenia after  
46 IS. A US nationwide inpatient sample study reported that 10% of hospitalized IS patients have comorbid  
47 cancer, 16% of them with gastrointestinal cancer and 1% with PDAC, and that this association may be  
48 on the rise <sup>24</sup>. Additionally, within two years after IS, another 2% to 4% of patients receive a new cancer  
49 diagnosis <sup>25-27</sup>. Within the SASKit study we aim to identify biomarkers to predict outcome after IS in  
50 terms of general health state (i.e. sarcopenia, deterioration of clinical performance, cognitive  
51 functioning, frailty) and quality of life, as well as (co-)morbidity, as we do for the PDAC cohort.  
52  
53  
54  
55  
56

57 ***Ischemic stroke, known biomarkers and clinical scores.*** In an early study of 956 patients with acute IS,  
58 determinants of long-term mortality were age, obesity, cardiac arrhythmias, diabetes mellitus,  
59 coronary heart disease and organic brain syndrome at discharge from hospital; interestingly,  
60



1  
2  
3 hypercholesterolaemia and smoking did not affect long-term outcome <sup>28</sup>. More recent studies  
4 uniformly identified age and stroke severity, usually assessed on the NIHSS or similar scales, as  
5 biomarkers of long-term functional outcome and mortality after stroke <sup>29 30</sup>. Fibrinogen has been  
6 related to long-term outcome after stroke <sup>31 32</sup>. There have been conflicting data on the predictive  
7 value of serum bilirubin levels on the long term risk of cardiovascular disease. While some studies are  
8 in favor of a predictive value (e.g.: <sup>33-35</sup>), others are not (e.g.: <sup>36</sup>). Also, CRP levels have been reported  
9 to impact the functional long-term outcome after IS <sup>37</sup>, and early neurological deterioration after IS has  
10 been related to decreasing albumin levels, elevated CRP and fibrinogen levels <sup>38</sup>. Potential biomarkers  
11 for occult cancer in IS patients include elevated D-dimers, fibrinogen, and CRP; infarction in multiple  
12 vascular territories; and poor nutritional status <sup>39</sup>. Interestingly, IS patients with elevation of at least  
13 two of the following coagulation-related serum markers, that is, D-dimer, prothrombin fragment 1.2,  
14 thrombin-antithrombin complex and fibrin monomer, in the post-acute phase of stroke, were more  
15 likely to have occult cancer or recurrent stroke during follow-up for 1.4±0.8 years <sup>40</sup>. In another study  
16 of acute IS patients, high D-dimer levels at admission were independently associated with recurrent  
17 stroke and all-cause mortality during follow-up for up to 3 years <sup>41</sup>. These findings underpin the idea  
18 of shared risk factors for unfavorable outcomes in IS as well as cancer and they suggest that there may  
19 be coagulation-related biomarkers indicating an early stage of carcinogenesis or stroke (see also Box  
20 2). Nevertheless, the clinical biomarkers that currently exist for predicting outcome are limited in their  
21 performance and clinical utility, and there is a need to overcome the limitations of current predictive  
22 models <sup>42</sup>.

---

**Box 1: Aging and cellular senescence.** Extra lifetime gained over the last century led to the widespread  
23 emergence of age-related diseases that are rarely seen in younger people. Older patients are thus  
24 more likely to display several comorbidities, which makes treatment difficult and expensive. Over the  
25 last years, strong evidence has accumulated that the presence of senescent cells (i.e. non-dividing,  
26 arrested but metabolically active cells that escape apoptosis) is causally involved in diseases such as  
27 atherosclerosis, cancer, fibrosis, pancreatitis, osteoarthritis, Alzheimer disease and metabolic  
28 disorders <sup>43 44</sup>. Evidence that senescent cells are not only correlated with aging and diseases, but are  
29 instead causally involved, comes from recent studies, which transplanted senescent cells from old into  
30 young mice <sup>45</sup>. This resulted in persistent functional impairment as well as spread of cellular senescence  
31 to host tissues. Another strong line of evidence comes from experiments that actually removed  
32 senescent cells from aged mice by *senolytics* <sup>45-47</sup>. In each case an increase in lifespan and a delay of  
33 typical age related diseases was observed. Most recently, the results of human pilot trials of putative  
34 senolytic treatments in case of idiopathic pulmonary fibrosis and osteoarthritis have been reported.  
35 One team <sup>48</sup> treated idiopathic pulmonary fibrosis patients with dasatinib and quercetin and  
36 demonstrated safety as well as notable improvements in some physical abilities. Furthermore, a  
37 human phase-1 study demonstrated that a senolytic compound, which was applied locally in patients  
38 with osteoarthritis of the knee, was safe and well-tolerated <sup>49</sup>. A clinically meaningful improvement in  
39 several measures, including pain, function, as well as modulation of certain senescence-associated  
40 secretory phenotype (SASP) factors and disease-related biomarkers was observed after a single dose.

---

**Box 2: Cellular senescence and the comorbidity of cancer and vascular events.** Some cancers such as  
41 PDAC can trigger vascular events by hyper-coagulation, reflecting Trousseau's syndrome first reported  
42 150 years ago <sup>11</sup>. In turn, strong associations between coagulation, cellular senescence and the SASP  
43 were demonstrated recently <sup>50</sup>. While cellular senescence can suppress PDAC and cancerous  
44 proliferation in general, it also triggers tumor progression by fostering inflammatory processes,  
45 including the SASP, while on the other hand, after ischemic stroke, it attenuates recovery <sup>51-55</sup>. For both



1  
2  
3 diseases, causal influences can be traced back to molecular determinants: PAI-1 (also known as  
4 SERPINE1 and part of the SASP) is involved in cancer-triggered thromboembolism<sup>52 54</sup> and stroke  
5 recovery in animals<sup>56</sup>. Other proteins involved in cellular senescence, specifically inflammatory  
6 cytokines such as IL6, and the lesser known osteopontin and gelsolin, are also markers for both PDAC  
7 and stroke<sup>57-60</sup>. The cyclin-dependent kinase CDK5<sup>61</sup> is implicated in the progression of PDAC as well  
8 as in the recovery from stroke<sup>55 62</sup>. Moreover, apart from being genetic risk factors<sup>63 64</sup>, the most  
9 prominent drivers of cellular senescence (p16/CDKN2A and p21/CDKN1A) also promote PDAC  
10 progression<sup>65</sup> and endothelial embolic and arteriosclerotic mechanisms of stroke<sup>66</sup>. Finally, two small-  
11 molecule interventions into cellular senescence, fisetin and quercetin, are both potential treatments  
12 of both PDAC and stroke. In case of stroke, the blood-brain-barrier is passed by quercetin which  
13 improves stroke outcome<sup>67</sup>. In case of PDAC it was observed that quercetin inhibits pancreatic cancer  
14 growth *in-vitro* and *in-vivo*<sup>68</sup>. Fisetin is found in various fruits (especially strawberries) and it is  
15 chemically similar to quercetin, with strong putative senolytic effects, extending lifespan of mice even  
16 when intervention with fisetin started only at an advanced age<sup>69</sup>. In a study involving nude mice  
17 implanted with prostate cancer cells, treatment with fisetin significantly retarded tumor growth<sup>70</sup>.  
18 Also, in case of lung cancer, there is evidence for the beneficial effects of fisetin. One study showed  
19 that fisetin provides protection against benzo(a)pyrene [B(a)P]-induced lung carcinogenesis in albino  
20 mice<sup>71</sup> and another *in vivo* study demonstrated the synergistic effects of fisetin and cyclophosphamide  
21 in reducing the growth of lung carcinoma in mice<sup>72</sup>. Several other studies have also demonstrated its  
22 anticarcinogenic, neurotrophic and anti-inflammatory effects that are beneficial in numerous diseases,  
23 including pancreatic cancer and stroke<sup>73</sup>.

---

## 31 Methods

32  
33 The presentation is based on the reporting recommendations for tumor marker prognostic studies  
34 (REMARK), that is, items (1) – (11) of the REMARK checklist<sup>74</sup>.

### 36 Study design

37  
38 The SASKit (“Senescence-Associated Systems diagnostics Kit for cancer and stroke”) study is designed  
39 as a prospective, observational, cohort study to identify biomarkers for disease deterioration in  
40 patients with PDAC or with IS and, specifically, for the (co-)morbidity of these diseases including  
41 vascular events and sarcopenia following the diagnosis of PDAC as well as cancer and cognitive decline  
42 following IS. All patients will be treated for their diseases in accordance with current guidelines or  
43 therapy standards and at the physician's discretion. Due to the observational study design, regular  
44 treatment of the patient is not affected apart from sampling blood (20 to 80 ml at up to 7 time-points  
45 over the next years). Assessment of disease deterioration will be based on standardized clinical  
46 performance measurements, and patient reported outcomes based on questionnaires (see below for  
47 details). Additionally, data from clinical charts and information from the general practitioner will be  
48 collected. The SASKit study is divided into two subtrials with a common control group, both featuring  
49 essentially the same outcomes, predictor measurements and data analysis approaches.

### 53 Patient and Public Involvement

54  
55 It was not possible to involve patients or the public in the design of the study.

### 57 Characteristics of participants (patients and controls)

58  
59 In the first subtrial (PDAC-subtrial), patients with an initial diagnosis of PDAC in locally advanced or  
60 metastatic stage without previous systemic therapy will be considered for enrollment, whereas

1  
2  
3 patients with a (thromboembolic) IS of the supratentorial brain region within the past 5 to 10 days,  
4 with a definitive brain infarction volume >10 ml in an assessment by magnetic resonance imaging (MRI)  
5 will be considered for the second subtrial (IS-subtrial). Except for some explorative analyses, the  
6 subtrials will be analyzed separately.  
7

8  
9 Within both subtrials, eligible as controls are those without PDAC or IS and with no other malignant  
10 disease or other (hemorrhagic) stroke during the past two years. Potential controls will be recruited  
11 from persons who have lived in the same household as the patient within the last 2 years, have a  
12 maximum age difference of 12 years and are neither brothers nor sisters (i.e. spouses, second-degree  
13 relatives or friends). The controls are selected so that the age and gender structure approximately  
14 reflects the age and gender distribution of the patients. Therefore, the age and gender of the patients  
15 will be continuously recorded, and the controls selected in such a way that their frequency distribution  
16 of gender at any time corresponds approximately to that of the currently recruited patients.  
17  
18

19 The following criteria lead to exclusion from participation in the study for both patients and controls,  
20 *at time of recruitment*:

- 21  
22
- 23 ● previous or current medical tumor therapy
  - 24 ● other cancer within the past 2 years
  - 25 ● previous stroke with persistent deficit
  - 26 ● myocardial infarction within the past 2 years
  - 27 ● therapeutic anticoagulation within the past 2 years for longer than 1 month
  - 28 ● pre-existing dementia
  - 29 ● chronic heart failure stage NYHA IV
  - 30 ● terminal renal insufficiency with hemodialysis
  - 31 ● known HIV infection
  - 32 ● known active hepatitis C
  - 33 ● pregnancy
  - 34 ● age < 18 years.
- 35  
36  
37  
38  
39  
40  
41  
42

43 Both subtrials will be implemented according to the same standardized protocol. After written  
44 informed consent of each participant, patients and controls will be followed up at 3, 12, 24, 36 and 48  
45 months after their inclusion in the trial, whenever possible. The PDAC-subtrial includes an additional  
46 time-point for examinations at 6 months after inclusion, given that mortality due to PDAC is expected  
47 to be accelerated as compared to IS.  
48  
49

50 The study is expected to start in the second quarter of 2020 and will finish with the last participant's  
51 follow up at 48 months. Until that time, we expect that 50 PDAC patients, 50 IS patients, and 50  
52 controls participated in the trial. The study will be conducted at the Rostock University Medical Center  
53 (UMR), Germany at Clinic III - Hematology, Oncology, Palliative Medicine and at the Department of  
54 Neurology; the institutions of the other co-authors are supporting the study in a variety of ways. The  
55 study protocol has been approved by the ethics committee of the UMR. The study is registered at  
56 German Clinical Trials Register (DRKS00021184) and will be conducted following ICH-GCP.  
57  
58

59 [General health- and disease-related and demographic data](#)

1  
2  
3 General data of the study participants will be recorded at the beginning of the study (“month 0”) and  
4 consist of the following: age, sex, BMI, temperature, blood pressure, heart rate (ECG). Furthermore,  
5 through interviews the following additional data will be recorded: vascular risk factors (arterial  
6 hypertension, diabetes, hyperlipidaemia, smoking habits), history of vascular events (stroke,  
7 myocardial infarction, venous or arterial thromboembolism), atrial fibrillation, history of cancer,  
8 current medication, surgery or blood transfusions in the past three months and vascular or cancerous  
9 events affecting any first degree relatives. These data may provide influential factors for explorative  
10 analyses, or be employed to interpret and discuss the results of the study.  
11  
12

### 13 Blood sampling

14  
15 Blood sampling will be done in a standardized fashion, that is, fasting and between 8 and 10 am, for all  
16 assays. Routine blood parameters will be recorded at the time-points described above (months 0 to  
17 48). These consist of differential blood count, INR (International normalized ratio of prothrombin time),  
18 partial thromboplastin time, D-dimers, fibrinogen, factor XII, albumin, bilirubin, high-sensitive CRP,  
19 CA19-9, cholesterol, and HbA1c.  
20  
21

22 Experimental blood analysis (PAI-1 and omics) will be done for patients at month 0 in case of PDAC, at  
23 month 0 or at month 3 in case of stroke (where the 3-month time point is taken if it reflects a better  
24 state of the patient as described by the NIHSS), and furthermore at month 3 in case of PDAC, and at  
25 month 12 in case of stroke. For controls, the experimental blood analysis will be carried out at month  
26 0 and at month 12, assuming that for these, data do not change much in the 3 months after baseline.  
27 The justification for taking the better state in case of stroke is the maximization of differences with the  
28 12 months follow-up data. In terms of practicality (being able to calculate a biomarker signature  
29 sooner), however, the state at month 0 should be selected for all stroke patients. Since the blood  
30 sample will be taken pre-processed and frozen at month 0 in all cases, we are in principle able to  
31 perform the experimental blood analysis for all stroke patients at month 0, and we can do this analysis  
32 in retrospect if deemed necessary. We also take blood of PDAC patients at month 12, to have the  
33 option to do an experimental blood analysis if deemed useful. In the following we will refer to the  
34 *baseline* time-point (month 0, or month 3 in cases of stroke patients that improved) and the *landmark*  
35 time-point (month 3 for PDAC patients and month 12 for stroke patients and controls). The  
36 experimental blood analysis is done earlier for PDAC because of high expected mortality within the  
37 first year.  
38  
39  
40  
41

42 The experimental blood analysis includes PAI-1 (see *Box 2*) as well as high-throughput (omics) analyses,  
43 that is, transcriptomics and proteomics analysis in T-cells and proteomics of serum. T cells are of  
44 interest because these were reported to carry the strongest signal with respect to cellular senescence,  
45 based on the marker p16<sup>75</sup>. We intend to measure gelsolin and osteopontin as well, provided that  
46 sufficiently standardized assays become available in due time; the blood collected for this  
47 measurement shall otherwise be used to measure cytokines/chemokines such as IL6, IL8 and TNF $\alpha$ ,  
48 which are part of the SASP, by ELISA assays. At time of writing, we do not yet have reliable estimates  
49 on the amount of blood cells still available for measuring protein expression, so an antibody-based  
50 protein array (in case of low amounts), or mass spectrometry (in case of sufficiently high amounts) will  
51 be used alternatively. For the blood serum, we intend to use the same protein measurement method.  
52 In the default case of a protein array, we plan to use the novel but dedicated “Senescence Associated  
53 Secretory Phenotype (SASP) Antibody Sampler Kit” (consisting of approx. 10 SASP-related proteins  
54 being measured; Cell Signaling Technology) for both cellular and serum proteomics. Further  
55 exploratory molecular analyses not (yet) funded but permitted based on the ethics approval include  
56 the following: single-cell analyses of blood, methylation assays for calculating epigenetic clocks<sup>76</sup>,  
57  
58  
59  
60

1  
2  
3 genetics by SNP array or whole-genome sequencing, and telomere length. A separate ethics approval  
4 was granted for an optional skin biopsy; skin microbiome analyses are planned as well.  
5

6 Blood sample processing for the experimental analysis will be performed according to standard  
7 operating procedures (SOP) at the research laboratory of Clinic III - Hematology, Oncology, Palliative  
8 Medicine. The procedures include flow cytometric control of the sampling quality including distribution  
9 of cell types and vitality as performed in routine diagnostics. Isolation of peripheral blood mononuclear  
10 cells (PBMCs) will also be performed following the SOP used by the laboratory in routine diagnostics.  
11 T-Cell separation will be performed according to an established work flow based on magnetic bead  
12 purification via Miltenyi MACS following manufacturer's instructions. T cell fraction purity as well as  
13 vitality will then be verified by flow cytometric analyses as described above. Nucleic acid isolation as  
14 well as protein isolation will be further performed according to the SOP of the research laboratory  
15 performed using column separation (Qiagen, Hilden Germany). RNA integrity values (RIN) will be  
16 analysed using an Agilent Scientific Instruments Bioanalyzer as instructed by the manufacturer. RIN  
17 values above 6 will qualify for RNAseq or Clariom D Array analyses; for RNAseq average reads per  
18 sample will be set at approx. 40 x 10e6.  
19  
20  
21  
22

### 23 Clinical performance measurements and patient-reported outcomes

24 At baseline and at each follow-up, handgrip strength ("grip strength" for short) is measured using a  
25 digital hand dynamometer (Jamar Plus). The test is performed while sitting comfortably, shoulder  
26 adducted, elbow placed on the tabletop and flexed to 90 degrees, with the forearm and wrist in a  
27 neutral position<sup>77</sup>. The highest value of three measurements of maximal isometric contraction of the  
28 dominant hand, or if paralyzed due to IS, contraction of the unaffected hand, is documented in kg.  
29 Further, the following clinical performance measurements are evaluated by the study physician or  
30 study nurse according to standard protocols: ECOG Performance Status (ECOG PS)<sup>78</sup>, modified Rankin  
31 Scale (mRS)<sup>79</sup>, Canadian Study on Health & Aging Clinical Frailty Scale (CSHA-CFS)<sup>80</sup>, NIH-Stroke Scale  
32 (NIHSS)<sup>81</sup>, Montreal Cognitive Assessment (MOCA)<sup>82</sup>. All raters are certified for the applicable scores  
33 (mRS, NIHSS, MOCA). Patient-reported outcomes (measured by questionnaires) are the following: EQ-  
34 5D-5L and EQ-VAS (generic evaluation of QoL in 5 domains and overall on a visual analog scale)<sup>83</sup>,  
35 HADS-D (evaluation of anxiety and depression)<sup>84</sup>, WHODAS 2.0 (WHO Disability Assessment Schedule)  
36<sup>85</sup>, and, for patients with PDAC, FACIT-Pal (evaluating QoL with focus on palliative symptoms and needs)  
37<sup>86, 87</sup>. All questionnaires are administered following the suppliers' instructions.  
38  
39  
40  
41

### 42 Follow up data

43 Apart from the clinical and patient-reported outcomes, further follow-up data are BMI, temperature,  
44 blood pressure, heart rate (ECG), atrial fibrillation, current medication, tumor treatment, comorbidity  
45 (any vascular or cancer event), hospital admissions or palliative care. Additionally, based on clinical  
46 charts and information from the general practitioner, we will record medication, (co-)morbidity and  
47 mortality. Just like the general health- and disease-related and demographic data recorded at time of  
48 recruitment, these data may provide influential factors for explorative analyses, or be employed to  
49 interpret and discuss the results of the study.  
50  
51  
52

### 53 Endpoints

54 In both subtrials, the primary endpoint is a composite measure of "disease deterioration" defined as  
55 the first occurrence within a follow-up interval of at least one of the following.  
56

- 57 a. Sarcopenia, measured by grip strength less than 27 kg for males and less than 16 kg for females  
58 (according to the revised European consensus, EWGSOP2,<sup>1</sup>).  
59  
60

- 1  
2  
3 b. Deterioration of clinical performance, that is, of the ECOG PS by at least two points (PDAC-  
4 subtrial), or of the mRS by at least one point (IS-subtrial).  
5  
6 c. Deterioration of QoL, described as a reduction of the EQ-5D-5L by at least 0.07 in the index  
7 score, **and** deterioration of at least 7 points in the EQ-VAS (ranging from 0-100).  
8

9 Deterioration will be considered between baseline (month 0) and the respective follow-up  
10 investigation. As described above, for patients with IS who have improved their condition (measured  
11 by NIHSS) within the first 3 months, this time point (month 3) will be used as a baseline instead. Item  
12 (a) is the deterioration from “no sarcopenia” to “probable sarcopenia” as defined by current consensus  
13 <sup>1</sup>. Grip strength has been widely used for assessing muscle strength, which is currently used as the  
14 most reliable measure of muscle function, loss of which indicating sarcopenia <sup>1</sup>. ECOG PS is established  
15 in describing the general condition of patients with cancer, whereas mRS is established in patients with  
16 stroke. Death is reflected by both scores as ECOG PS of 5 or mRS of 6, and it will always consider death  
17 from any cause. The EQ-5D-5L evaluates QoL in five dimensions (mobility, self-care, usual activity,  
18 pain/discomfort, and anxiety/depression), all relevant for patients with PDAC and IS. Furthermore, it  
19 is a generic score so that results will be comparable for different diseases (as recently described in  
20 patients with stroke <sup>88</sup>) and for the general population <sup>89</sup>). Even though disease-specific scores might  
21 evaluate symptom burden in even more detail, the EQ-5D-5L was recently shown to be comparable to  
22 QoL scores developed specifically for pulmonary embolism and deep vein thrombosis (that is, PEmb-  
23 QoL, VEINES-QOL/Sym and PACT-Q2) in terms of acceptability, validity and responsiveness <sup>90</sup>. A clinical  
24 deterioration in EQ-5D-5L is described as a minimal important difference in the range from 0.07 to 0.09  
25 index points and in VAS from 7 to 10 <sup>91</sup> which is the basis for the definition of item (c). Controls reach  
26 their endpoint by the same definition as the subcohort for which they serve as control; in any  
27 integrative analysis of both subtrials, a deterioration of the mRS by at least one point will be used as  
28 the criterion (instead of ECOG PS), because stroke patients in general have a slower deterioration than  
29 PDAC patients, and controls naturally have the slowest expected deterioration.  
30  
31  
32  
33  
34  
35

36 The primary composite endpoint and all secondary endpoints will be evaluated in a first analysis, based  
37 on data obtained until summer 2021, and in a second analysis, based on data obtained until summer  
38 2023, and in a third analysis at the end of the study. The second analysis may be delayed until data of  
39 90% of the study participants are available (at least including the month 12 follow up) and it may then  
40 constitute the “main” analysis of the study.  
41

42 The following secondary endpoints are evaluated:

- 43  
44  
45 ● each component of the primary endpoint (separately);  
46 ● occurrence of disease-specific (co-)morbidity, as follows  
47 ○ new vascular events (stroke, myocardial infarction, venous or arterial  
48 thromboembolism), specifically in patients with PDAC;  
49 ○ new cancer, specifically in patients with IS;  
50 ○ probable sarcopenia (based on grip strength);  
51 ○ cognitive decline (deterioration of MOCA by 3 points from best value at baseline);  
52 ● frailty, defined as a CSHA-CFS level of 6, 7, or 8;  
53 ● all-cause mortality.  
54  
55

56 Further, a sum-score summarizing all measurements of phenotypic variables (grip strength, clinical  
57 performance measurements, comorbid events, mortality) will be considered as a surrogate for “aging”,  
58 normalizing all continuous-scaled components in order to obtain a common scale with an average of  
59 zero and standard deviation of one. The components of the sum-score will all be given equal weight.  
60



## Predictors

While all phenotypic features (grip strength, clinical performance, patient reported outcomes, comorbid events, mortality) are contributing to the definition of endpoints (as dependent variables/parameters), all routine and experimental blood features (PAI-1, omics) are considered to be potential predictors; these are also called the independent variables/parameters. This delineation is justified by (a) the paradigm that (clinical) relevance is tied to high-level phenotypes describing health and survival, specifically including QoL <sup>2</sup>, and (b) the goal of developing a “senescence-associated systems diagnostics kit” that includes a careful selection of biomarkers contributing, as much as possible, also to molecular-mechanistic insights into PDAC, IS and their (co-)morbidity, which we hypothesize to be related to cellular senescence and aging. Age and gender will be included as mandatory covariates (also termed confounders, that is, predictors which we do not aim to explore, or which we wish to improve upon) in all statistical models. Further covariates are smoking, the baseline NIHSS score in case of IS, as well as locally-advanced vs metastatic PDAC and modality of treatment in case of PDAC. As described, the successful predictors identified by our study, following the statistical analyses outlined below, are called biomarkers; we wish to stress that these are only *candidates* for the ultimate goal of *clinically validated biomarkers*; in particular, they still need to be validated in further studies (based, e.g., on other cohorts). A set of biomarkers is also called a biomarker signature.

## Blinding and pseudonymization

No blinding will be done during the study. However, the primary composite endpoint will be documented without subjective influence due to standardized definitions. Thus, detection bias will be kept at a minimal extent. Furthermore, information bias will be minimized as we will use simple measurements, which are applied in daily practice or are self-reported and easy to perform (e.g. EQ-5D-5L). The rigorous inclusion of all eligible patients within the recruitment period will help to minimize selection bias. All patient data are pseudonymized to all investigators except for the attending physician and study nurse. Since all major data analyses are based on known information about the outcomes (e.g., supervised machine learning with cross-validation), the data analysis will also be performed based on the pseudonymized data. Protection of personal and clinical data of all patients and controls will follow all relevant legal regulations.

## Sample size

No formal sample size calculation was performed a-priori for this observational study. The prevalence of PDAC combined with the requirement to complete the study within a reasonable timeframe implied a target of 50 patients per group (PDAC, IS and control group). Nevertheless, a power analysis revealed that a sample size of 50 patients will have 80% power to detect a significant difference by a non-parametric Wilcoxon statistic between an AUC of 0.75 for a particular biomarker signature compared to the null hypothesis value of 0.5 at a significance level of 5% under the assumption that about three times as many patients will reach the primary endpoint, compared to patients who will not reach the primary endpoint <sup>92</sup>.

## Data Analysis Plan

**General considerations:** The guiding criteria for biomarker identification in the SASKit study are the maximization of the predictive signal, clinical relevance/utility, biomedical/molecular/clinical interpretability, and practicality/cost. Given the relatively low number of participants in this in-depth study, to maximize the signal for the endpoints and predictors given as outlined above, we must aim

1  
2  
3 to use all available information. Regarding endpoints, whenever possible, we thus wish to consider the  
4 (censored) time-to-event information inherent in the baseline and follow-up examinations, and in the  
5 mortality data. The primary endpoint was defined to integrate expected clinical utility and maximum  
6 signal. In defining the (secondary) endpoints, we considered an array of clinically relevant single  
7 endpoints as well as a sum-score of all phenotypic measurements; we hypothesize that the latter  
8 carries the largest amount of signal. Given the small sample, we cannot set aside an extra validation  
9 dataset. (For the predictors considered to be covariates/confounders, please see the section on  
10 “Predictors”, above.)  
11  
12  
13

14  
15 **Data quality assessment and cleaning:** The need for (and the amount of) data cleaning cannot easily  
16 be estimated beforehand; we plan to follow the MarkAGE guidelines<sup>93</sup> to deal with missing values,  
17 and to detect and rectify outliers and batch artefacts.  
18

19  
20 **Predictor/Feature integration:** Regarding predictors (features), we first need to remember that we  
21 measure at baseline (at months 0 or 3) and at one landmark (main followup, that is, at months 3 or  
22 12). While use of baseline features is unrestricted, use of landmark features is, of course, restricted to  
23 predict outcomes after the landmark. Further, we need to handle the high dimensionality of the omics  
24 features. Here, upfront feature integration, e.g., by averaging measurements as described below, is  
25 considered preferable specifically for the high-dimensional omics data, for the following reasons.  
26  
27

- 28 1) A small feature space allows for an easier understanding and interpretation, see, e.g.,<sup>94</sup>.
- 29 2) Integrated features can be used as input for both the standard biostatistics and the standard  
30 machine learning parts of the analysis.
- 31 3) Use of few features is more time-tested than newer methods featuring the joint calculation of  
32 the prediction model and the selection of the features, albeit the latter are quite often claimed  
33 to be superior by their developers.
- 34 4) Naturally, feature integration avoids multicollinearity and overfitting, and multiple testing is  
35 less of an issue. This counters the “curse of dimensionality” and “de-noises” the data towards  
36 better prediction performance<sup>94 95</sup>.
- 37 5) Feature integration allows the handling of feature heterogeneity, which in our case refers to  
38 routine blood measurements as well as various omics data types.
- 39 6) In the *explorative* analyses, systems biology modelling and the parallelogram approach are  
40 both supposed to deliver further small sets of integrated, highly informative features, which  
41 may, e.g., dominate systems behaviour, or which are believed to translate well from animal  
42 models to humans (see below).  
43  
44  
45  
46  
47  
48

49 While most features will be available for the baseline and the landmark time-point, utilizing baseline  
50 data is clinically more useful, simply because the prediction for the endpoint is available much earlier.  
51 Nevertheless, in the explorative analyses, we will investigate the predictive power of *changes* in  
52 feature measurements from baseline to landmark, given that such changes may be more informative  
53 about future disease deterioration (and other endpoints) than just baseline values.  
54  
55

56  
57 **Specific omics data feature integration:** Notably, we face a heterogeneous “multi-view” dataset,  
58 usually referred to as “multi-omics”. Our feature integration approach (see above) is also known as a  
59 “late integration” type of analysis, implying that measurements for different omics data types are  
60 reduced early on to activation scores for pathways or subnetworks that are then integrated at a “late”



1  
2  
3 level. To calculate the activation scores for subnetworks, we use, by default, the  
4 ExprEssence/FocusHeuristics *linkscore*<sup>96 97</sup>, taking the links (gene/protein interactions) from a  
5 functional interaction network defaulting to STRING. Our experience with the *linkscore* motivates us  
6 to include this method as one of the approaches proposed for feature integration in the following,  
7 influencing the calculation of up to 10 features on which the standard biostatistics and machine  
8 learning shall be based. Specifically, we take the average expression measurement for all patients  
9 (as a list of expression values, one per gene) and the average for all controls (as a list of expression  
10 values, one per gene) to calculate a *linkscore* for each STRING interaction, and assemble a  
11 “condensed” network including all interactions with a *linkscore* in that percentile for which the 50  
12 highest-scoring interactions are shown. These interactions form subnetworks. We then take the  
13 average *linkscore* for each subnetwork as the subnetwork activation score. Alternative methods  
14 such as *keypathwayminer* will be used in the exploratory analyses, see below. For the pathways (such  
15 as KEGG), we will calculate pathway activation scores using Gene Set Variation Analysis (GSVA)<sup>98</sup>. This  
16 method calculates pathway activation scores from expression data, is suited for use with microarray  
17 as well as RNAseq data and performed strongly in a recent benchmarking analysis<sup>99</sup>. The GSVA-based  
18 pathway activation scores can subsequently be compared between patients and controls in the same  
19 way as normal gene expression data, calculating, for each pathway, a fold-change of the pathway  
20 activation scores between patients and controls. Here, we average over all patients and over all  
21 controls, respectively, using the *limma* R package and adjusting for age and gender of the individual  
22 patient/control pathway activation. An example of this approach is given in the GSVA publication,  
23 where differential pathway activation was identified between acute lymphoblastic lymphoma and  
24 mixed-lineage lymphoma<sup>98</sup>. The major downside of feature integration may be information loss;  
25 subsequent statistical and machine-learning-based analyses receive only a tiny fraction of the amount  
26 of information that is available in total.

27 Gene expression data (transcriptomics) will be our preferred omics data type. Nevertheless, proteins  
28 are closer to the phenotype than transcripts, so we wish to not ignore these. Therefore, we prepare to  
29 deal with both kinds of proteome data that we may expect (see “Experimental blood analyses”, above),  
30 as follows.

- 31 1. Large-scale data, likely based on mass spectrometry, in the order of hundreds or more proteins  
32 that can be identified and measured in all the conditions investigated differentially.
- 33 2. Small-scale data, likely based on antibody arrays, in the order of tens or less.

34 Except for the raw data preprocessing depending on the platform, once log-fold changes describing  
35 differential expression are established, we thus expect to handle the large-scale proteome data  
36 essentially the same as the transcriptomics data, and the small-scale proteome data similarly to the  
37 blood routine data, for cells and serum alike. Overall, the omics data are expected to come along three  
38 main coordinates, that is,

- 39 1. as blood cell transcriptomics and proteomics as well as serum proteomics;
- 40 2. longitudinal in time (for baseline and landmark); and
- 41 3. for PDAC, IS and control.

42 All coordinates can be exploited for differential analyses, even though the PDAC and IS data will be  
43 analyzed separately except for some integrative *explorative* analyses (see below). In the *explorative*  
44 analyses, the *longitudinal* transcriptomics of the patients and controls will also be analyzed together,  
45 see below. For the standard biostatistics and machine learning analyses, we plan to employ 5  
46 approaches to feature integration, each yielding a shortlist of 5 integrated features, as follows.

- 1) **(5 features)** A first shortlist of features will consist of the following expert selection from the routine blood measurements (incl. PAI-1): *neutrophil-lymphocyte-ratio, fibrinogen, high-sensitive C-reactive protein, albumin* and *PAI-1*.
- 2) **(5 features)** For the cellular gene expression measurements, we use ExprEssence/FocusHeuristics (see above) to calculate *the top-5 subnetworks scoring highest*.
- 3) **(5 features)** Again for the cellular gene expression measurements, we use GSVA (see above) to calculate the top-5 most strongly changing pathways as features.
- 4) + 5) **(10 features)**
  - a) In case of dealing with large-scale serum proteomics data, we proceed as in (2) + (3);
  - b) In case of dealing with small-scale serum proteomics data, we proceed as follows:
    - i) if the number of features measured successfully is in the order of 10, we refrain from any processing;
    - ii) if the number of features is in the order of around 10-100, we select the 10 features with the smallest p-values indicating differences between the mean values of patient and control, based on a t-test.

For genomic features as per (2), the feature measurements for an individual patient or control will then be the average linkscores of the 5 selected subnetworks. For genomic features as per (3), the feature measurements for each patient/control will be the GSVA scores of the 5 selected pathways. By construction, we expect the resulting features to reflect the up/downregulation of disease-related transcripts/proteins or pathways/subnetworks. Using the GSVA-based integrated features as input to the biostatistical analyses employing Cox proportional hazard models, we are in fact closely following the “Survival analysis in ovarian carcinoma” example as described in the GSVA publication<sup>98</sup>. Regarding the expert selection from the routine blood measurements, we are aware that some of these features may be considered to have an almost trivial relationship to outcome prediction for the diseases we study; e.g. fibrinogen may correlate strongly with the size of the stroke-damaged brain area and may thus be considered a covariate. However, to our knowledge, none of these features are validated clinical biomarkers, and it is quite possible that a combination of simple biomarkers is key to the best possible prediction. We selected the *neutrophil-lymphocyte-ratio* specifically because it is cheap to measure; it is, however, like many other blood-based features, easily influenced by acute infection.

**Exploratory feature integration:** Apart from the FocusHeuristics/ExprEssence *linkscore*, we employ alternatives such as *keypathwayminer*<sup>100</sup>. Further, we calculate pathway activation scores for the following senescence-related KEGG pathways, which include PAI-1 (see the Introduction) but do not refer to a specific disease, as of February 2020: *Cellular senescence, HIF-1 signaling pathway, p53 signaling pathway, Apelin signaling pathway, Hippo signaling pathway, Complement and coagulation cascades*. “Early integration” by, e.g., first averaging transcript and protein expression on a single-gene basis, is also planned.

**Choice of data analysis methods for biomarker discovery:** We will consider two main approaches of data analysis, one motivated by statistical methods, the other by machine learning approaches. While this delineation may ultimately be meaningless, we consider that regression is the core ingredient of the former, while supervised learning characterizes the latter. We will apply “standard” methods (mostly in biostatistics) and explore novel approaches (mostly in machine learning; preserving signal implies a focus on *supervised* approaches in this case). Data analysis for biomarker *discovery* trials in a *clinical* setting is usually described with a biostatisticians’ mindset, who also developed methods to

1  
2  
3 cope with the high dimensionality of omics data (see below). On the other hand, the challenges of  
4 omics data also spurred the recent publication of many methods adopting machine learning, which  
5 however did not yet make it into clinical trial analysis routine, but which we wish to test (see below).  
6 We will focus on methods readily available in SAS or as R packages. Notably, the correct choice of  
7 method depends in part on known unknowns such as the strength of the signal (incl. the amount of  
8 missing data) in the routine blood measurements and the omics.  
9  
10

11  
12 **Prediction model quality measures:** Unlike intervention trials with their highly standardized aim of  
13 establishing a statistically significant superiority (or non-inferiority) of one intervention compared to  
14 another (or to standard of care), observational biomarker trials are a more recent development with  
15 fewer precisely quantified criteria of success, and a stronger need to consider the effect size: even if a  
16 biomarker signature enables a significant improvement in predicting an outcome, raising the accuracy  
17 of the prediction, say, from 70% to 75% may not be clinically meaningful, depending on prevalence of  
18 the condition to be predicted, the cost of the biomarker measurement, etc. We thus aim to identify  
19 biomarkers making a maximum of *difference* in prediction accuracy, if we are able to compare to  
20 established scores (see also below). For the biostatistics part, the concordance statistics (c-index) will  
21 be used as an overall measure of predictive accuracy, and time-dependent ROC curves and AUC will  
22 be used to summarize the predictive accuracy at different cut-off points in time. For the machine  
23 learning part, the cross-validated accuracy and AUC/c-index, following<sup>94</sup>, are used, and to take care of  
24 a potential Simpson's paradox we will either analyse the data stratified by gender, or we will add such  
25 an analysis and check for consistency. More generally, to investigate the role of confounders (and, if  
26 necessary, to correct for these) in the machine learning part, we wish to use the permutation technique  
27 described<sup>101</sup>. We expect that we can identify a set of biomarkers that affords an accuracy of 75% or  
28 more or an AUC of 0.75 or more in correctly predicting the primary endpoint with a precision of +/-  
29 12%<sup>102</sup>. This estimate of precision is based on half the width of a 95% confidence interval (CI) for a  
30 probability of 75%, by extension of item 6 of the tables of Sorzano et al<sup>102</sup>, which shows precision up  
31 to a sample size of N=30.  
32  
33  
34  
35  
36  
37  
38

39 **Standard biostatistical analyses:** A Cox proportional hazards regression model adjusted for age and  
40 gender will be used to estimate the hazard ratio (HR) and corresponding 95% CI to predict the primary  
41 composite endpoint separately within the PDAC cohort and IS cohort. The 5 shortlists of 5 features  
42 (see above) will be providing the canonical predictors, analyzed together. For selection of the most  
43 important features that might be related to the primary endpoint we will use a procedure proposed  
44 by Sauerbrei et al.<sup>103</sup>, as follows. First, 100 bootstrap samples will be generated. Then, a multivariate  
45 Cox proportional hazards regression model with backward elimination with selection level of 0.05 will  
46 be fitted to each replication of the original data set. In a second step features with a relative selection  
47 frequency of 30% or less over all bootstrap samples will be eliminated. In a third step each feature  $X_i$   
48 for which the hypothesis of independence in combination with a feature  $X_j$  can be rejected will be  
49 eliminated if  $X_i$  is less important when  $X_j$  is included in the model, or if it does not gain importance  
50 when  $X_j$  is excluded from the model. All remaining features will be included in the final model.  
51 Graphical and numerical methods will be performed to establish the validity of the proportionality  
52 assumption<sup>104</sup> in the final model. Results will be reported as p-values, HRs and corresponding 95%-CIs.  
53 A p-value of  $p \leq 0.05$  will be interpreted as indicating statistical significance. From the final model a risk  
54 score will be calculated by multiplying the individual feature measurement of a patient with the  
55 estimated regression coefficient of each feature. The c-index will be used as an overall measure of  
56 predictive accuracy of the resulting score, a time-dependent ROC curve and AUC will be used to  
57  
58  
59  
60

1  
2  
3 summarize the predictive accuracy of the score at specific times. All secondary endpoints will be  
4 evaluated using the same approach as for the primary endpoint except for the sum-score used as a  
5 surrogate for “aging”. For this endpoint, a linear mixed effects model with random intercept and spatial  
6 power covariance structure will be fitted to the data to estimate the progression of “aging”. The  
7 covariance structure is chosen to reflect the unequal intervals of follow up investigations. Model  
8 assumptions and model fit will be checked by visual inspection of residuals, and influence diagnostics.  
9 Missing values will be taken into account by a likelihood-based approach within the framework of  
10 mixed linear models with the assumption that missing values occur at random. Results will be reported  
11 as p-value assessed at a level of significance of 5% accompanied by the value of the test statistic and  
12 degrees of freedom. In addition, 95% CIs for the progression (slope) will be provided.  
13  
14  
15

16  
17 **Additional exploratory biostatistical analyses:** Again, the primary composite endpoint as well as all  
18 secondary endpoints will be evaluated separately within the PDAC cohort and IS cohort of the  
19 respective sub-trials. In a first approach, univariate Cox proportional hazard models adjusted for age  
20 and gender will be calculated for each omics feature (R package *survival*) using a cut-off of 0.05 on the  
21 false discovery rate. In a second approach, all omics features will be simultaneously considered in a  
22 multivariate Cox model, adjusted for age and gender. Towards this aim, a component-wise likelihood-  
23 based boosting algorithm proposed by Binder and Schumacher 2008 <sup>105</sup> (R package *CoxBoost*) will be  
24 used to develop a biomarker signature.  
25  
26  
27

28  
29 **Standard machine learning:** For the machine learning part, the primary outcome and all secondary  
30 outcomes give rise to an assignment of predictor/feature lists to survival times, one such list per study  
31 participant, for which biomarkers are then learned in a supervised fashion. As described, in the  
32 standard analyses, feature integration (see above) will precede the actual calculation of the model  
33 (“deep” learning approaches that take in “all” features are part of the *exploratory* analyses, see below).  
34 In the same way as the standard biostatistics analyses, the same 5 shortlists of 5 features each (see  
35 above) will be providing the canonical predictors, analyzed together. Exploiting time-to-event  
36 information, we will employ random survival forests (RSF) as described by <sup>106</sup> with the following  
37 advantages.  
38  
39  
40

- 41 1. RSF can now be considered a time-tested approach, and it was the subject of a recent  
42 extensive review <sup>65</sup> and of a systematic comparison with LASSO approaches in the case without  
43 feature selection (see item 7 of the tables of Pi *et al* <sup>107</sup>, for its competitive performance which  
44 is not reflected in their abstract).
- 45 2. RSF can also work on essentially all features, without a preceding feature integration/selection  
46 step, and then be compared, in the explorative machine learning analyses described below, to  
47 survival support vector machines (SSVM) and to a novel method Path2Surv that “conjointly”  
48 performs feature selection and model training, see <sup>94</sup>.
- 49 3. RSF was recently compared to Cox-nnet <sup>108</sup>, a neural network approach which we consider as  
50 very promising for the *exploratory* part, see also below.
- 51 4. RSF offers a considerable degree of interpretability, given that RSFs are derived from decision  
52 trees.
- 53 5. RSF is considered “completely data driven and thus independent of model assumptions” and  
54 “in case of high dimensional data, limitations of univariate regression approaches such as  
55 overfitting, unreliable estimation of regression coefficients, inflated standard errors or  
56 convergence problems do not apply” <sup>65</sup>.
- 57  
58  
59  
60

1  
2  
3 In the machine learning part, we calculate accuracy and AUC/c-index using cross-validation to make  
4 the best use of our limited sample size, following the setup of <sup>94</sup> and <sup>107</sup> (who, however, set aside  
5 separate validation datasets).  
6

7  
8 **Additional exploratory machine learning:** Apart from the more time-tested standard machine learning  
9 described above, we will also explore methods that were proposed recently, for which it is less  
10 straightforward to tell whether these methods are fit-for-purpose in our case, even though they are  
11 usually claimed to be superior by their developers based on some test/validation data sets. Specifically,  
12 as mentioned above, we expect to test Path2Surv and SSVM <sup>94</sup> as well as Cox-nnet <sup>108</sup> (without prior  
13 feature integration); the latter in particular promises a high degree of interpretability. We further  
14 explore CNet (employing the censored-data variant), for interpretable biomarkers. We also plan to  
15 employ the PASNet <sup>109</sup>, SurvivalNet <sup>110</sup> and SVRc <sup>70</sup> packages. The longitudinal transcriptomics of the  
16 patients and the controls may also be analyzed integratively based on the “optimal discovery  
17 procedure” <sup>111</sup>, considering, however, that landmark feature data can only be used to predict events  
18 after the landmark. Finally, we will map the differential omics data onto a human “healthspan pathway  
19 map” <sup>112</sup>, that is, a set of clusters/pathways based on health-related genetic data that we assembled  
20 recently.  
21  
22

23  
24  
25  
26 **Explorative systems biology modelling, explorative parallelogram approach and transfer learning:**  
27 As mentioned, systems biology modelling and parallelogram <sup>113</sup> <sup>114</sup> extrapolation are supposed to  
28 deliver small sets of highly informative features, by contributing features that are dominating model  
29 behaviour or that are shown to translate from the SASKit animal model data. Given the comparatively  
30 small number of study participants (but in-depth measurements), we also wish to explore “transfer  
31 learning”, which aims to utilize large amounts of public knowledge in the form of latent variables.  
32 Specifically, we plan to use, and wish to develop further, the Multiplier <sup>115</sup> approach motivated by the  
33 analysis of rare-disease data. Multiplier utilizes the RNASeq-based recount2 compendium, and apart  
34 from the functional network and pathway data that we use in the feature selection part, this  
35 compendium is expected to be our main source of biological knowledge that enters the calculations  
36 for biomarker discovery.  
37  
38  
39

40  
41  
42 **Miscellaneous exploratory approaches and discovery of diagnostic biomarkers:** We will also use  
43 unsupervised machine learning to generate descriptive multi-omics correlation networks, as they were  
44 most recently employed by <sup>116</sup>, there supplemented by linear mixed effects models using (un-  
45 )restricted maximum likelihood approaches; in this very recent biomarker discovery trial of similar  
46 design as ours, but with many more longitudinal omics measurement time-points than ours, we could  
47 not identify other biomarker discovery methods being used. If genetic data become available, we will  
48 include these in some analyses; specifically, we will investigate the added value of *expression*  
49 *quantitative trait loci* (eQTL) analyses. PDAC and IS data will be analyzed together in some integrative  
50 *exploratory* analyses. In that case, the occurrence of specific endpoints will be evaluated according to  
51 the group membership (PDAC or IS). This means that in addition to the biomarker signature, a group  
52 variable, indicating PDAC or IS patients, will be included in the analysis, to assess the difference in the  
53 progression of the respective endpoints between PDAC and IS patients. We also wish to compare PDAC  
54 and IS patient data to data of healthy controls (adjusted for age and gender) by means of logistic  
55 regression models with the aim of identifying candidate biomarkers for the diagnosis of the respective  
56 disease; we then specifically investigate the association of these diagnostic biomarker candidates with  
57 cellular senescence and other aging-related processes (see also the next paragraph).  
58  
59  
60



1  
2  
3  
4 **Further analyses, and comparison with existing biomarkers and biomarker signatures:** Towards the  
5 end, we will investigate the overlap for the various biomarker identification approaches we employed,  
6 assuming that the most frequently found biomarkers may be the most robust and valid ones.  
7 Moreover, we will compare with existing biomarkers and signatures. Regarding the prediction of  
8 vascular events, we will specifically calculate the Khorana and related scores<sup>17</sup> for comparison, and  
9 report the difference in performance. Further, for all biomarkers we find, we will check their  
10 association with cellular senescence, by manual inspection, literature investigation, comparison to  
11 CellAge<sup>117</sup> and the SASP Atlas<sup>50</sup> or by formal enrichment analyses if the number of biomarkers is  
12 sufficiently large to do this in a meaningful way. Also, in a final step, we plan to identify and filter out  
13 the biomarkers that are volatile in the controls. In addition, a comparison of the biomarker profiles  
14 before and after the co-morbid event is aimed for. Finally, for publicly available data of other trials  
15 with a sufficient overlap with our predictors, we will use these as validation datasets.

## 21 Discussion

### 23 Limitations

25 Arguably, the most serious limitation of the SASKit study is the low number of participants. We  
26 mentioned above that in the 4-year-time-frame of the entire study, at the Rostock University Medical  
27 Center we cannot expect to recruit many more than the 50 PDAC patients to be included in this study;  
28 we could recruit more stroke patients and more controls, but given the call for proposals that allowed  
29 this exploratory (not confirmatory) study to be applied for and funded, we considered that within a  
30 limited budget, in-depth omics characterization, animal models (to be detailed in a follow up  
31 publication) and a comprehensive data analysis plan including systems biology modelling were  
32 important aspects of our study that we did not want to exclude.

35 The two most obvious risks to the main goal of finding good biomarkers for the primary outcome based  
36 on the standard data analysis are the following. First, we found it hard to estimate the distribution of  
37 events as defined by the primary outcome; we cannot exclude that too many events take place already  
38 at the start of the study, or until the first follow-up, specifically in the PDAC subtrial, limiting the  
39 amount of information available to the subsequent time-to-event analyses. Then again, had we  
40 defined the primary outcome more conservatively, there would have been a chance that not enough  
41 events happen until the end of the study. Second, we could not identify role-model publications  
42 reporting results of biomarker explorations that made use of machine learning methods, except for,  
43 to some extent,<sup>116</sup> so that we enter unknown territory to some degree. The two most obvious risks  
44 to our goal of investigating the role of cellular senescence in the (co-)morbidity of PDAC and IS could  
45 be an insufficient prevalence of co-morbid events, and the complex role of treatment in case of PDAC,  
46 where additional cellular senescence is most likely triggered by therapeutic intervention<sup>118</sup>. Then  
47 again, all molecular high-throughput analyses are essentially explorative and we are open to  
48 discovering biomarkers of disease that do *not* relate to any of our pre-specified hypotheses.

### 53 Implications

54 We designed the SASKit study to synergistically deliver upon a couple of aims that we consider to be  
55 of relevance for specific disease prognosis and treatment as well as for primary, secondary and tertiary  
56 prevention. Employing clinical performance measurements and patient-reported outcomes, we aim  
57 for clinical relevance and we suggest that prognostic biomarker signatures for general health and QoL  
58 are perhaps more important than (progression-free) survival, although there is much more data about  
59 the latter than the former. Moreover, good disease treatment options are still lacking for PDAC as well

as for stroke, and the more we find cellular senescence implicated in disease deterioration, at least in a subgroup of patients with a specific biomarker signature, the more confidently we can suggest, and further explore, seno-therapeutic interventions for these two diseases.

Notably, we are in the process of starting a parallel human study testing, in healthy elderly people, interventions into cellular senescence, based on *food* rich in seno-interventional compounds, and we expect that many aspects of the study design presented herein will be adopted in that parallel study. That study will also investigate aging- and senescence-related outcomes, and as such it can be seen as a test of a cautious yet potentially very effective approach to primary prevention; if the *diagnostic* biomarkers we find in the SASKit study relate to cellular senescence, this observation would constitute further evidence for (cautious) seno-interventions, moving towards a kind of universal approach of disease prevention by tackling fundamental aging-related processes (see Boxes 1 and 2).

Secondary prevention, aiming to reduce the impact of a disease that has already occurred, can ultimately be supported by the SASKit study, if we can demonstrate, and (in follow up studies) confirm, a distinctive role of cellular senescence (and/or other aging-related processes such as inflammation/inflammaging<sup>119</sup>) in disease deterioration as defined here. Finally, evidence for tertiary prevention by seno-therapeutic intervention, aiming to attenuate the impact of an ongoing disease, is also an option based on how accurate, relevant and specific our biomarkers will be.

Last but not least, we expect that the in-depth molecular analyses that we wish to conduct will provide mechanistic insights into the etiology of the diseases we study here, which we just see as models for the investigation of the fundamental role of aging in general and cellular senescence in particular in disease and dysfunction.

#### Abbreviations:

|        |   |
|--------|---|
| AUC    | Area Under the Curve  |
| BMI    | Body Mass Index   |
| CA19-9 | Carbohydrate Antigen  |
| CEA    | Carcinoembryonic antigen  |
| CI     | Confidence interval   |
| CRP    | C-reactive protein  |
| ECOG   | Eastern Cooperative Oncology Group                                  |
| HR     | Hazard ratio  |
| INR    | International normalized ratio                                      |
| IS     | Ischemic Stroke   |
| LDH    | Lactate dehydrogenase   |
| NIHSS  | NIH-Stroke Scale  |
| NYHA   | New York Heart Association  |
| PDAC   | Pancreatic Ductal Adenocarcinoma                                    |
| PS     | Performance status  |
| QoL    | Quality of Life   |
| ROC    | Receiver-Operator Characteristic                                    |
| RSF    | Random survival forests   |
| SASKit | Senescence-Associated Systems diagnostics Kit for cancer and stroke |
| SASP   | Senescence Associated Secretory Phenotype                           |

#### Contributorship



1  
2  
3 All authors contributed important intellectual content to the study design and/or the writing of the  
4 study protocol.  
5

#### 6 Conflict of Interest

7  
8 Dr. Walter reports personal fees from Ipsen Pharma, grants and personal fees from Merz Pharma,  
9 personal fees from Allergan, personal fees from Bristol-Myers Squibb, personal fees from Daiichi  
10 Sankyo, personal fees from Bayer Vital, personal fees from Boehringer Ingelheim, personal fees from  
11 Pfizer, personal fees from Thieme, and personal fees from Elsevier Press, all outside the submitted  
12 work. The other authors have nothing to disclose.  
13

#### 14 Funding

15  
16 We acknowledge the financial support by the Federal Ministry of Education and Research (BMBF) of  
17 Germany for the SASKit study (FKZ 01ZX1903A). The funder had no role in the design of the study.  
18

#### 19 Data sharing statement

20  
21 No data available.  
22

#### 23 References

- 24 1. Cruz-Jentoft AJ, Bahat G, Bauer J, et al. Sarcopenia: revised European consensus on definition and  
25 diagnosis. *Age Ageing* 2019;48(1):16-31. doi: 10.1093/ageing/afy169 [published Online First:  
26 2018/10/13]
- 27 2. Fuellen G, Jansen L, Cohen AA, et al. Health and Aging: Unifying Concepts, Scores, Biomarkers and  
28 Pathways. *Aging and Disease* 2019;10(4):883-900.
- 29 3. Collaborators GBDPC. The global, regional, and national burden of pancreatic cancer and its  
30 attributable risk factors in 195 countries and territories, 1990-2017: a systematic analysis for  
31 the Global Burden of Disease Study 2017. *Lancet Gastroenterol Hepatol* 2019;4(12):934-47.  
32 doi: 10.1016/S2468-1253(19)30347-4
- 33 4. Kleeff J, Korc M, Apte M, et al. Pancreatic cancer. *Nat Rev Dis Primers* 2016;2:16022. doi:  
34 10.1038/nrdp.2016.22 [published Online First: 2016/05/10]
- 35 5. Llop E, P EG, Duran A, et al. Glycoprotein biomarkers for the detection of pancreatic ductal  
36 adenocarcinoma. *World J Gastroenterol* 2018;24(24):2537-54. doi: 10.3748/wjg.v24.i24.2537  
37 [published Online First: 2018/07/03]
- 38 6. Carrato A, Falcone A, Ducreux M, et al. A Systematic Review of the Burden of Pancreatic Cancer in  
39 Europe: Real-World Impact on Survival, Quality of Life and Costs. *J Gastrointest Cancer*  
40 2015;46(3):201-11. doi: 10.1007/s12029-015-9724-1 [published Online First: 2015/05/15]
- 41 7. Cruz-Jentoft AJ, Sayer AA. Sarcopenia. *Lancet* 2019;393(10191):2636-46. doi: 10.1016/S0140-  
42 6736(19)31138-9 [published Online First: 2019/06/07]
- 43 8. Taieb J, Pointet AL, Van Laethem JL, et al. What treatment in 2017 for inoperable pancreatic cancers?  
44 *Ann Oncol* 2017;28(7):1473-83. doi: 10.1093/annonc/mdx174 [published Online First:  
45 2017/05/02]
- 46 9. Menapace LA, Peterson DR, Berry A, et al. Symptomatic and incidental thromboembolism are both  
47 associated with mortality in pancreatic cancer. *Thromb Haemost* 2011;106(2):371-8. doi:  
48 10.1160/TH10-12-0789 [published Online First: 2011/06/30]
- 49 10. Grilz E, Posch F, Konigsbrugge O, et al. Association of Platelet-to-Lymphocyte Ratio and Neutrophil-  
50 to-Lymphocyte Ratio with the Risk of Thromboembolism and Mortality in Patients with Cancer.  
51 *Thromb Haemost* 2018;118(11):1875-84. doi: 10.1055/s-0038-1673401 [published Online  
52 First: 2018/10/09]
- 53 11. Bonnerot M, Humbertjean L, Mione G, et al. Cerebral ischemic events in patients with pancreatic  
54 cancer: A retrospective cohort study of 17 patients and a literature review. *Medicine*  
55  
56  
57  
58  
59  
60

- (Baltimore) 2016;95(26):e4009. doi: 10.1097/MD.0000000000004009 [published Online First: 2016/07/02]
12. Navi BB, Reiner AS, Kamel H, et al. Association between incident cancer and subsequent stroke. *Ann Neurol* 2015;77(2):291-300. doi: 10.1002/ana.24325 [published Online First: 2014/12/05]
  13. Varki A. Trousseau's syndrome: multiple definitions and multiple mechanisms. *Blood* 2007;110(6):1723-9. doi: 10.1182/blood-2006-10-053736 [published Online First: 2007/05/15]
  14. Grilz E, Marosi C, Konigsbrugge O, et al. Association of complete blood count parameters, d-dimer, and soluble P-selectin with risk of arterial thromboembolism in patients with cancer. *J Thromb Haemost* 2019;17(8):1335-44. doi: 10.1111/jth.14484 [published Online First: 2019/05/18]
  15. Liu Z, Jin K, Guo M, et al. Prognostic Value of the CRP/Alb Ratio, a Novel Inflammation-Based Score in Pancreatic Cancer. *Ann Surg Oncol* 2017;24(2):561-68. doi: 10.1245/s10434-016-5579-3 [published Online First: 2016/09/22]
  16. Haas M, Heinemann V, Kullmann F, et al. Prognostic value of CA 19-9, CEA, CRP, LDH and bilirubin levels in locally advanced and metastatic pancreatic cancer: results from a multicenter, pooled analysis of patients receiving palliative chemotherapy. *J Cancer Res Clin Oncol* 2013;139(4):681-9. doi: 10.1007/s00432-012-1371-3
  17. van Es N, Di Nisio M, Cesarman G, et al. Comparison of risk prediction scores for venous thromboembolism in cancer patients: a prospective cohort study. *Haematologica* 2017;102(9):1494-501. doi: 10.3324/haematol.2017.169060 [published Online First: 2017/05/28]
  18. Khorana AA, Kuderer NM, Culakova E, et al. Development and validation of a predictive model for chemotherapy-associated thrombosis. *Blood* 2008;111(10):4902-7. doi: 10.1182/blood-2007-10-116327 [published Online First: 2008/01/25]
  19. Kruger S, Haas M, Burkl C, et al. Incidence, outcome and risk stratification tools for venous thromboembolism in advanced pancreatic cancer - A retrospective cohort study. *Thromb Res* 2017;157:9-15. doi: 10.1016/j.thromres.2017.06.021
  20. Faille D, Bourrienne MC, de Raucourt E, et al. Biomarkers for the risk of thrombosis in pancreatic adenocarcinoma are related to cancer process. *Oncotarget* 2018;9(41):26453-65. doi: 10.18632/oncotarget.25458 [published Online First: 2018/06/15]
  21. Stahmeyer J, Stubenrauch S, Geyer S, et al. The frequency and timing of recurrent stroke—an analysis of routine health insurance data. *Dtsch Arztebl Int* 2019;116:711-7.
  22. Ryan AS, Ivey FM, Serra MC, et al. Sarcopenia and Physical Function in Middle-Aged and Older Stroke Survivors. *Arch Phys Med Rehabil* 2017;98(3):495-99. doi: 10.1016/j.apmr.2016.07.015 [published Online First: 2016/08/18]
  23. Scherbakov N, von Haehling S, Anker SD, et al. Stroke induced Sarcopenia: muscle wasting and disability after stroke. *Int J Cardiol* 2013;170(2):89-94. doi: 10.1016/j.ijcard.2013.10.031 [published Online First: 2013/11/16]
  24. Sanossian N, Djabiras C, Mack WJ, et al. Trends in cancer diagnoses among inpatients hospitalized with stroke. *J Stroke Cerebrovasc Dis* 2013;22(7):1146-50. doi: 10.1016/j.jstrokecerebrovasdis.2012.11.016 [published Online First: 2012/12/19]
  25. Uemura J, Kimura K, Sibazaki K, et al. Acute stroke patients have occult malignancy more often than expected. *Eur Neurol* 2010;64(3):140-4. doi: 10.1159/000316764 [published Online First: 2010/07/30]
  26. Cocho D, Gendre J, Boltès A, et al. Predictors of occult cancer in acute ischemic stroke patients. *J Stroke Cerebrovasc Dis* 2015;24(6):1324-8. doi: 10.1016/j.jstrokecerebrovasdis.2015.02.006 [published Online First: 2015/04/18]
  27. Selvik HA, Thomassen L, Bjerkreim AT, et al. Cancer-Associated Stroke: The Bergen NORSTROKE Study. *Cerebrovasc Dis Extra* 2015;5(3):107-13. doi: 10.1159/000440730 [published Online First: 2015/12/10]
  28. Weitbrecht WU, Kirchhoff D. [Long-term prognosis of cerebral infarct in comparison with a normal population]. *Versicherungsmedizin* 1995;47(2):46-9. [published Online First: 1995/04/01]

- 1
  - 2
  - 3
  - 4
  - 5
  - 6
  - 7
  - 8
  - 9
  - 10
  - 11
  - 12
  - 13
  - 14
  - 15
  - 16
  - 17
  - 18
  - 19
  - 20
  - 21
  - 22
  - 23
  - 24
  - 25
  - 26
  - 27
  - 28
  - 29
  - 30
  - 31
  - 32
  - 33
  - 34
  - 35
  - 36
  - 37
  - 38
  - 39
  - 40
  - 41
  - 42
  - 43
  - 44
  - 45
  - 46
  - 47
  - 48
  - 49
  - 50
  - 51
  - 52
  - 53
  - 54
  - 55
  - 56
  - 57
  - 58
  - 59
  - 60
29. Meyer S, Verheyden G, Brinkmann N, et al. Functional and motor outcome 5 years after stroke is equivalent to outcome at 2 months: follow-up of the collaborative evaluation of rehabilitation in stroke across Europe. *Stroke* 2015;46(6):1613-9. doi: 10.1161/STROKEAHA.115.009421 [published Online First: 2015/05/09]
30. Drozdowska BA, Singh S, Quinn TJ. Thinking About the Future: A Review of Prognostic Scales Used in Acute Stroke. *Front Neurol* 2019;10:274. doi: 10.3389/fneur.2019.00274 [published Online First: 2019/04/06]
31. Pedersen A, Stanne TM, Redfors P, et al. Fibrinogen concentrations predict long-term cognitive outcome in young ischemic stroke patients. *Res Pract Thromb Haemost* 2018;2(2):339-46. doi: 10.1002/rth2.12078 [published Online First: 2018/07/27]
32. Swarowska M, Polczak A, Pera J, et al. Hyperfibrinogenemia predicts long-term risk of death after ischemic stroke. *J Thromb Thrombolysis* 2014;38(4):517-21. doi: 10.1007/s11239-014-1122-1 [published Online First: 2014/08/12]
33. Perlstein TS, Pande RL, Creager MA, et al. Serum total bilirubin level, prevalent stroke, and stroke outcomes: NHANES 1999-2004. *Am J Med* 2008;121(9):781-88 e1. doi: 10.1016/j.amjmed.2008.03.045 [published Online First: 2008/08/30]
34. Choi Y, Lee SJ, Spiller W, et al. Causal Associations Between Serum Bilirubin Levels and Decreased Stroke Risk: A Two-Sample Mendelian Randomization Study. *Arterioscler Thromb Vasc Biol* 2020;40(2):437-45. doi: 10.1161/ATVBAHA.119.313055 [published Online First: 2019/12/06]
35. Zhong P, Wu D, Ye X, et al. Association of circulating total bilirubin level with ischemic stroke: a systemic review and meta-analysis of observational evidence. *Ann Transl Med* 2019;7(14):335. doi: 10.21037/atm.2019.06.71 [published Online First: 2019/09/03]
36. Jorgensen ME, Torp-Pedersen C, Finer N, et al. Association between serum bilirubin and cardiovascular disease in an overweight high risk population from the SCOUT trial. *Nutr Metab Cardiovasc Dis* 2014;24(6):656-62. doi: 10.1016/j.numecd.2013.12.009 [published Online First: 2014/02/19]
37. Wang L, Li Y, Wang C, et al. C-reactive Protein, Infection, and Outcome After Acute Ischemic Stroke: A Registry and Systematic Review. *Curr Neurovasc Res* 2019;16(5):405-15. doi: 10.2174/1567202616666191026122011 [published Online First: 2019/11/19]
38. Martin AJ, Price CI. A Systematic Review and Meta-Analysis of Molecular Biomarkers Associated with Early Neurological Deterioration Following Acute Stroke. *Cerebrovasc Dis* 2018;46(5-6):230-41. doi: 10.1159/000495572 [published Online First: 2018/12/06]
39. Navi BB, Iadecola C. Ischemic stroke in cancer patients: A review of an underappreciated pathology. *Ann Neurol* 2018;83(5):873-83. doi: 10.1002/ana.25227 [published Online First: 2018/04/11]
40. Ellis D, Rangaraju S, Duncan A, et al. Coagulation markers and echocardiography predict atrial fibrillation, malignancy or recurrent stroke after cryptogenic stroke. *Medicine (Baltimore)* 2018;97(51):e13830. doi: 10.1097/MD.00000000000013830 [published Online First: 2018/12/24]
41. Nezu T, Kitano T, Kubo S, et al. Impact of D-dimer levels for short-term or long-term outcomes in cryptogenic stroke patients. *J Neurol* 2018;265(3):628-36. doi: 10.1007/s00415-018-8742-x [published Online First: 2018/01/27]
42. Chaudhary D, Abedi V, Li J, et al. Clinical Risk Score for Predicting Recurrence Following a Cerebral Ischemic Event. *Front Neurol* 2019;10:1106. doi: 10.3389/fneur.2019.01106 [published Online First: 2019/11/30]
43. Yanai H, Fraifeld VE. The role of cellular senescence in aging through the prism of Koch-like criteria. *Ageing Res Rev* 2018;41:18-33. doi: 10.1016/j.arr.2017.10.004 [published Online First: 2017/11/07]
44. Gonzalez-Meljem JM, Apps JR, Fraser HC, et al. Paracrine roles of cellular senescence in promoting tumourigenesis. *Br J Cancer* 2018;118(10):1283-88. doi: 10.1038/s41416-018-0066-1 [published Online First: 2018/04/20]

- 1  
2  
3 45. Xu M, Pirtskhalava T, Farr JN, et al. Senolytics improve physical function and increase lifespan in  
4 old age. *Nat Med* 2018;24(8):1246-56. doi: 10.1038/s41591-018-0092-9 [published Online  
5 First: 2018/07/11]  
6  
7 46. Baker DJ, Childs BG, Durik M, et al. Naturally occurring p16(Ink4a)-positive cells shorten healthy  
8 lifespan. *Nature* 2016;530(7589):184-9. doi: 10.1038/nature16932 [published Online First:  
9 2016/02/04]  
10  
11 47. Baar MP, Brandt RMC, Putavet DA, et al. Targeted Apoptosis of Senescent Cells Restores Tissue  
12 Homeostasis in Response to Chemotoxicity and Aging. *Cell* 2017;169(1):132-47 e16. doi:  
13 10.1016/j.cell.2017.02.031 [published Online First: 2017/03/25]  
14  
15 48. Justice JN, Nambiar AM, Tchkonina T, et al. Senolytics in idiopathic pulmonary fibrosis: Results from  
16 a first-in-human, open-label, pilot study. *EBioMedicine* 2019 doi:  
17 10.1016/j.ebiom.2018.12.052  
18  
19 49. UNITY. UNITY Biotechnology Reports Promising Topline Data from Phase 1 First-in-human Study of  
20 UBX0101 in Patients with Osteoarthritis of the Knee, 2019.  
21  
22 50. Tanaka T, Biancotto A, Moaddel R, et al. Plasma proteomic signature of age in healthy humans.  
23 *Aging Cell* 2018;17(5):e12799. doi: 10.1111/accel.12799  
24  
25 51. Childs BG, Gluscevic M, Baker DJ, et al. Senescent cells: an emerging target for diseases of ageing.  
26 *Nat Rev Drug Discov* 2017;16(10):718-35. doi: 10.1038/nrd.2017.116 [published Online First:  
27 2017/07/22]  
28  
29 52. Hisada Y, Mackman N. Cancer-associated pathways and biomarkers of venous thrombosis. *Blood*  
30 2017;130(13):1499-506. doi: 10.1182/blood-2017-03-743211 [published Online First:  
31 2017/08/16]  
32  
33 53. Moir JA, White SA, Mann J. Arrested development and the great escape--the role of cellular  
34 senescence in pancreatic cancer. *Int J Biochem Cell Biol* 2014;57:142-8. doi:  
35 10.1016/j.biocel.2014.10.018 [published Online First: 2014/12/03]  
36  
37 54. Valenzuela CA, Quintanilla R, Moore-Carrasco R, et al. The Potential Role of Senescence As a  
38 Modulator of Platelets and Tumorigenesis. *Front Oncol* 2017;7:188. doi:  
39 10.3389/fonc.2017.00188 [published Online First: 2017/09/13]  
40  
41 55. Posada-Duque RA, Barreto GE, Cardona-Gomez GP. Protection after stroke: cellular effectors of  
42 neurovascular unit integrity. *Front Cell Neurosci* 2014;8:231. doi: 10.3389/fncel.2014.00231  
43 [published Online First: 2014/09/02]  
44  
45 56. Chan SL, Bishop N, Li Z, et al. Inhibition of PAI (Plasminogen Activator Inhibitor)-1 Improves Brain  
46 Collateral Perfusion and Injury After Acute Ischemic Stroke in Aged Hypertensive Rats. *Stroke*  
47 2018;49(8):1969-76. doi: 10.1161/STROKEAHA.118.022056 [published Online First:  
48 2018/07/12]  
49  
50 57. Garcia-Berrocoso T, Penalba A, Boada C, et al. From brain to blood: New biomarkers for ischemic  
51 stroke prognosis. *J Proteomics* 2013;94:138-48. doi: 10.1016/j.jprot.2013.09.005 [published  
52 Online First: 2013/09/26]  
53  
54 58. Mendioroz M, Fernandez-Cadenas I, Rosell A, et al. Osteopontin predicts long-term functional  
55 outcome among ischemic stroke patients. *J Neurol* 2011;258(3):486-93. doi: 10.1007/s00415-  
56 010-5785-z [published Online First: 2010/10/23]  
57  
58 59. Pan S, Chen R, Brand RE, et al. Multiplex targeted proteomic assay for biomarker detection in  
59 plasma: a pancreatic cancer biomarker case study. *J Proteome Res* 2012;11(3):1937-48. doi:  
60 10.1021/pr201117w [published Online First: 2012/02/10]  
61  
62 60. Poruk KE, Firpo MA, Scaife CL, et al. Serum osteopontin and tissue inhibitor of metalloproteinase 1  
63 as diagnostic and prognostic biomarkers for pancreatic adenocarcinoma. *Pancreas*  
64 2013;42(2):193-7. doi: 10.1097/MPA.0b013e31825e354d [published Online First:  
65 2013/02/15]  
66  
67 61. Alexander K, Yang HS, Hinds PW. Cellular senescence requires CDK5 repression of Rac1 activity.  
68 *Mol Cell Biol* 2004;24(7):2808-19. doi: 10.1128/mcb.24.7.2808-2819.2004 [published Online  
69 First: 2004/03/17]  
70



- 1  
2  
3 62. Feldmann G, Mishra A, Hong SM, et al. Inhibiting the cyclin-dependent kinase CDK5 blocks  
4 pancreatic cancer formation and progression through the suppression of Ras-Ral signaling.  
5 *Cancer Res* 2010;70(11):4460-9. doi: 10.1158/0008-5472.CAN-09-1107 [published Online First:  
6 2010/05/21]  
7  
8 63. Akinyemi R, Tiwari HK, Arnett DK, et al. APOL1, CDKN2A/CDKN2B, and HDAC9 polymorphisms and  
9 small vessel ischemic stroke. *Acta Neurol Scand* 2018;137(1):133-41. doi: 10.1111/ane.12847  
10 [published Online First: 2017/10/05]  
11  
12 64. Cremin C, Howard S, Le L, et al. CDKN2A founder mutation in pancreatic ductal adenocarcinoma  
13 patients without cutaneous features of Familial Atypical Multiple Mole Melanoma (FAMMM)  
14 syndrome. *Hered Cancer Clin Pract* 2018;16:7. doi: 10.1186/s13053-018-0088-y [published  
15 Online First: 2018/03/16]  
16  
17 65. Wang T, Notta F, Navab R, et al. Senescent Carcinoma-Associated Fibroblasts Upregulate IL8 to  
18 Enhance Prometastatic Phenotypes. *Mol Cancer Res* 2017;15(1):3-14. doi: 10.1158/1541-  
19 7786.MCR-16-0192 [published Online First: 2016/09/30]  
20  
21 66. Chen J, Huang X, Halicka D, et al. Contribution of p16INK4a and p21CIP1 pathways to induction of  
22 premature senescence of human endothelial cells: permissive role of p53. *Am J Physiol Heart*  
23 *Circ Physiol* 2006;290(4):H1575-86. doi: 10.1152/ajpheart.00364.2005 [published Online First:  
24 2005/10/26]  
25  
26 67. Tressera-Rimbau A, Arranz S, Eder M, et al. Dietary Polyphenols in the Prevention of Stroke.  
27 *Oxidative medicine and cellular longevity* 2017;2017:7467962. doi: 10.1155/2017/7467962  
28  
29 68. Angst E, Park JL, Moro A, et al. The flavonoid quercetin inhibits pancreatic cancer growth in vitro  
30 and in vivo. *Pancreas* 2013;42(2):223-9. doi: 10.1097/MPA.0b013e318264ccae  
31  
32 69. Yousefzadeh MJ, Zhu Y, McGowan SJ, et al. Fisetin is a senotherapeutic that extends health and  
33 lifespan. *EBioMedicine* 2018;36:18-28. doi: 10.1016/j.ebiom.2018.09.015  
34  
35 70. Khan FM, Zubek VB. Support Vector Regression for Censored Data (SVRC): A Novel Tool for Survival  
36 Analysis. Eighth IEEE International Conference on Data Mining. Pisa, Italy, 2008.  
37  
38 71. Ravichandran N, Suresh G, Ramesh B, et al. Fisetin, a novel flavonol attenuates benzo(a)pyrene-  
39 induced lung carcinogenesis in Swiss albino mice. *Food and chemical toxicology : an*  
40 *international journal published for the British Industrial Biological Research Association*  
41 2011;49(5):1141-7. doi: 10.1016/j.fct.2011.02.005  
42  
43 72. Touil YS, Seguin J, Scherman D, et al. Improved antiangiogenic and antitumour activity of the  
44 combination of the natural flavonoid fisetin and cyclophosphamide in Lewis lung carcinoma-  
45 bearing mice. *Cancer Chemother Pharmacol* 2011;68(2):445-55. doi: 10.1007/s00280-010-  
46 1505-8  
47  
48 73. Khan N, Syed DN, Ahmad N, et al. Fisetin: a dietary antioxidant for health promotion. *Antioxid*  
49 *Redox Signal* 2013;19(2):151-62. doi: 10.1089/ars.2012.4901  
50  
51 74. Altman DG, McShane LM, Sauerbrei W, et al. Reporting Recommendations for Tumor Marker  
52 Prognostic Studies (REMARK): explanation and elaboration. *PLoS Med* 2012;9(5):e1001216.  
53 doi: 10.1371/journal.pmed.1001216 [published Online First: 2012/06/08]  
54  
55 75. Liu Y, Sanoff HK, Cho H, et al. Expression of p16(INK4a) in peripheral blood T-cells is a biomarker of  
56 human aging. *Aging Cell* 2009;8(4):439-48. doi: 10.1111/j.1474-9726.2009.00489.x  
57  
58 76. Ward-Caviness CK, Huffman JE, Everett K, et al. DNA methylation age is associated with an altered  
59 hemostatic profile in a multiethnic meta-analysis. *Blood* 2018;132(17):1842-50. doi:  
60 10.1182/blood-2018-02-831347  
61  
62 77. Sousa-Santos AR, Amaral TF. Differences in handgrip strength protocols to identify sarcopenia and  
63 frailty - a systematic review. *BMC Geriatr* 2017;17(1):238. doi: 10.1186/s12877-017-0625-y  
64 [published Online First: 2017/10/19]  
65  
66 78. Oken MM, Creech RH, Tormey DC, et al. Toxicity and response criteria of the Eastern Cooperative  
67 Oncology Group. *Am J Clin Oncol* 1982;5(6):649-55. [published Online First: 1982/12/01]  
68  
69 79. van Swieten JC, Koudstaal PJ, Visser MC, et al. Interobserver agreement for the assessment of  
70 handicap in stroke patients. *Stroke* 1988;19(5):604-7. doi: 10.1161/01.str.19.5.604 [published  
71 Online First: 1988/05/01]

- 1
  - 2
  - 3
  - 4
  - 5
  - 6
  - 7
  - 8
  - 9
  - 10
  - 11
  - 12
  - 13
  - 14
  - 15
  - 16
  - 17
  - 18
  - 19
  - 20
  - 21
  - 22
  - 23
  - 24
  - 25
  - 26
  - 27
  - 28
  - 29
  - 30
  - 31
  - 32
  - 33
  - 34
  - 35
  - 36
  - 37
  - 38
  - 39
  - 40
  - 41
  - 42
  - 43
  - 44
  - 45
  - 46
  - 47
  - 48
  - 49
  - 50
  - 51
  - 52
  - 53
  - 54
  - 55
  - 56
  - 57
  - 58
  - 59
  - 60
80. Rockwood K, Song X, MacKnight C, et al. A global clinical measure of fitness and frailty in elderly people. *CMAJ* 2005;173(5):489-95. doi: 10.1503/cmaj.050051 [published Online First: 2005/09/01]
81. Lyden P, Brott T, Tilley B, et al. Improved reliability of the NIH Stroke Scale using video training. NINDS TPA Stroke Study Group. *Stroke* 1994;25(11):2220-6. doi: 10.1161/01.str.25.11.2220 [published Online First: 1994/11/01]
82. Nasreddine ZS, Phillips NA, Bedirian V, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *J Am Geriatr Soc* 2005;53(4):695-9. doi: 10.1111/j.1532-5415.2005.53221.x [published Online First: 2005/04/09]
83. Herdman M, Gudex C, Lloyd A, et al. Development and preliminary testing of the new five-level version of EQ-5D (EQ-5D-5L). *Qual Life Res* 2011;20(10):1727-36. doi: 10.1007/s11136-011-9903-x [published Online First: 2011/04/12]
84. Snaith RP, Zigmond AS. The hospital anxiety and depression scale. *Br Med J (Clin Res Ed)* 1986;292(6516):344. doi: 10.1136/bmj.292.6516.344 [published Online First: 1986/02/01]
85. Ustun TB, Chatterji S, Kostanjsek N, et al. Developing the World Health Organization Disability Assessment Schedule 2.0. *Bull World Health Organ* 2010;88(11):815-23. doi: 10.2471/BLT.09.067231 [published Online First: 2010/11/16]
86. Lyons KD, Bakitas M, Hegel MT, et al. Reliability and validity of the Functional Assessment of Chronic Illness Therapy-Palliative care (FACIT-Pal) scale. *J Pain Symptom Manage* 2009;37(1):23-32. doi: 10.1016/j.jpainsymman.2007.12.015 [published Online First: 2008/05/28]
87. Sewtz C, Muscheites W, Kriesen U, et al. Questionnaires measuring quality of life and satisfaction of patients and their relatives in a palliative care setting-German translation of FAMCARE-2 and the palliative care subscale of FACIT-Pal. *Ann Palliat Med* 2018;7(4):420-26. doi: 10.21037/apm.2018.03.17 [published Online First: 2018/06/05]
88. Golicki D, Niewada M, Karlinska A, et al. Comparing responsiveness of the EQ-5D-5L, EQ-5D-3L and EQ VAS in stroke patients. *Qual Life Res* 2015;24(6):1555-63. doi: 10.1007/s11136-014-0873-7 [published Online First: 2014/11/27]
89. Ludwig K, Graf von der Schulenburg JM, Greiner W. German Value Set for the EQ-5D-5L. *Pharmacoeconomics* 2018;36(6):663-74. doi: 10.1007/s40273-018-0615-8 [published Online First: 2018/02/21]
90. Chuang LH, Cohen AT, Agnelli G, et al. Comparison of quality of life measurements: EQ-5D-5L versus disease/treatment-specific measures in pulmonary embolism and deep vein thrombosis. *Qual Life Res* 2019;28(5):1155-77. doi: 10.1007/s11136-018-2081-3 [published Online First: 2019/01/05]
91. Pickard AS, Neary MP, Cella D. Estimation of minimally important differences in EQ-5D utility and VAS scores in cancer. *Health and quality of life outcomes* 2007;5:70. doi: 10.1186/1477-7525-5-70
92. Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. *Radiology* 1982;143(1):29-36. doi: 10.1148/radiology.143.1.7063747 [published Online First: 1982/04/01]
93. Baur J, Moreno-Villanueva M, Kotter T, et al. MARK-AGE data management: Cleaning, exploration and visualization of data. *Mech Ageing Dev* 2015;151:38-44. doi: 10.1016/j.mad.2015.05.007 [published Online First: 2015/05/26]
94. Dereli O, Oguz C, Gonen M. Path2Surv: Pathway/gene set-based survival analysis using multiple kernel learning. *Bioinformatics* 2019;35(24):5137-45. doi: 10.1093/bioinformatics/btz446 [published Online First: 2019/05/31]
95. Buzdin A, Sorokin M, Garazha A, et al. Molecular pathway activation - New type of biomarkers for tumor morphology and personalized selection of target drugs. *Semin Cancer Biol* 2018;53:110-24. doi: 10.1016/j.semcancer.2018.06.003 [published Online First: 2018/06/24]
96. Warsow G, Greber B, Falk SS, et al. ExprEssence--revealing the essence of differential experimental data in the context of an interaction/regulation net-work. *BMC Syst Biol* 2010;4:164. doi: 10.1186/1752-0509-4-164 [published Online First: 2010/12/02]

- 1  
2  
3 97. Ernst M, Du Y, Warsaw G, et al. FocusHeuristics - expression-data-driven network optimization and  
4 disease gene prediction. *Sci Rep* 2017;7:42638. doi: 10.1038/srep42638 [published Online  
5 First: 2017/02/17]  
6  
7 98. Hanzelmann S, Castelo R, Guinney J. GSVA: gene set variation analysis for microarray and RNA-seq  
8 data. *BMC Bioinformatics* 2013;14:7. doi: 10.1186/1471-2105-14-7 [published Online First:  
9 2013/01/18]  
10  
11 99. Geistlinger L, Csaba G, Santarelli M, et al. Toward a gold standard for benchmarking gene set  
12 enrichment analysis. *Brief Bioinform* 2020 doi: 10.1093/bib/bbz158 [published Online First:  
13 2020/02/07]  
14  
15 100. List M, Alcaraz N, Dissing-Hansen M, et al. KeyPathwayMinerWeb: online multi-omics network  
16 enrichment. *Nucleic Acids Res* 2016;44(W1):W98-W104. doi: 10.1093/nar/gkw373 [published  
17 Online First: 2016/05/07]  
18  
19 101. Neto E, Pratap A, Perumal T, et al. Using permutations to assess confounding in machine learning  
20 applications for digital health. *ArXiv* 2018; arXiv:1811.11920 or arXiv:1811.11920v1  
21  
22 102. Sorzano C, Tabas-Madrid D, Nunez F, et al. Sample Size for Pilot Studies and Precision Driven  
23 Experiments. *ArXiv* 2017; arXiv:1707.00222 or arXiv:1707.00222v2  
24  
25 103. Sauerbrei W, Schumacher M. A bootstrap resampling procedure for model building: application  
26 to the Cox regression model. *Stat Med* 1992;11(16):2093-109. doi: 10.1002/sim.4780111607  
27 [published Online First: 1992/12/01]  
28  
29 104. Lin DY. Cox regression analysis of multivariate failure time data: the marginal approach. *Stat Med*  
30 1994;13(21):2233-47. doi: 10.1002/sim.4780132105 [published Online First: 1994/11/15]  
31  
32 105. Binder H, Schumacher M. Allowing for mandatory covariates in boosting estimation of sparse  
33 high-dimensional survival models. *BMC Bioinformatics* 2008;9:14. doi: 10.1186/1471-2105-9-  
34 14 [published Online First: 2008/01/12]  
35  
36 106. Ishwaran H, Kogalur UB, Blackstone EH, et al. Random survival forests. *Ann Appl Stat*  
37 2008;2(3):841-60. doi: 10.1214/08-AOAS169  
38  
39 107. Pi L, Halabi S. Combined Performance of Screening and Variable Selection Methods in Ultra-High  
40 Dimensional Data in Predicting Time-To-Event Outcomes. *Diagn Progn Res* 2018;2 doi:  
41 10.1186/s41512-018-0043-4 [published Online First: 2018/11/06]  
42  
43 108. Ching T, Zhu X, Garmire LX. Cox-nnet: An artificial neural network method for prognosis prediction  
44 of high-throughput omics data. *PLoS Comput Biol* 2018;14(4):e1006076. doi:  
45 10.1371/journal.pcbi.1006076 [published Online First: 2018/04/11]  
46  
47 109. Hao J, Kim Y, Kim TK, et al. PASNet: pathway-associated sparse deep neural network for prognosis  
48 prediction from high-throughput data. *BMC Bioinformatics* 2018;19(1):510. doi:  
49 10.1186/s12859-018-2500-z  
50  
51 110. Yousefi S, Amrollahi F, Amgad M, et al. Predicting clinical outcomes from large scale cancer  
52 genomic profiles with deep survival models. *Sci Rep* 2017;7(1):11707. doi: 10.1038/s41598-  
53 017-11817-6  
54  
55 111. Bass A, Storey J. *bioRxiv* 2019 doi: 10.1101/571992  
56  
57 112. Moeller S, Saul N, Cohen AA, et al. Healthspan pathway maps in *C. elegans* and humans highlight  
58 transcription, proliferation/biosynthesis and lipids. *bioRxiv* 2018  
59  
60 113. Motwani HV, Frostne C, Tornqvist M. Parallelogram based approach for in vivo dose estimation  
of genotoxic metabolites in humans with relevance to reduction of animal experiments. *Sci  
Rep* 2017;7(1):17560. doi: 10.1038/s41598-017-17692-5  
114. Kienhuis AS, van de Poll MC, Wortelboer H, et al. Parallelogram approach using rat-human in vitro  
and rat in vivo toxicogenomics predicts acetaminophen-induced hepatotoxicity in humans.  
*Toxicol Sci* 2009;107(2):544-52. doi: 10.1093/toxsci/kfn237  
115. Taroni JN, Grayson PC, Hu Q, et al. MultiPLIER: A Transfer Learning Framework for Transcriptomics  
Reveals Systemic Features of Rare Disease. *Cell Syst* 2019;8(5):380-94 e4. doi:  
10.1016/j.cels.2019.04.003 [published Online First: 2019/05/24]



- 1  
2  
3 116. Schussler-Fiorenza Rose SM, Contrepolis K, Moneghetti KJ, et al. A longitudinal big data approach  
4 for precision health. *Nat Med* 2019;25(5):792-804. doi: 10.1038/s41591-019-0414-6  
5 [published Online First: 2019/05/10]  
6  
7 117. Avelar RA, Ortega JG, Tacutu R, et al. A Multidimensional Systems Biology Analysis of Cellular  
8 Senescence in Ageing and Disease. *bioRxiv* 2019  
9  
10 118. Demaria M, O'Leary MN, Chang J, et al. Cellular Senescence Promotes Adverse Effects of  
11 Chemotherapy and Cancer Relapse. *Cancer Discov* 2017;7(2):165-76. doi: 10.1158/2159-  
12 8290.CD-16-0241 [published Online First: 2016/12/17]  
13  
14 119. Fulop T, Larbi A, Dupuis G, et al. Immunosenescence and Inflamm-Aging As Two Sides of the Same  
15 Coin: Friends or Foes? *Front Immunol* 2017;8:1960. doi: 10.3389/fimmu.2017.01960  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For peer review only

# BMJ Open

## Towards biomarkers for outcomes after pancreatic ductal adenocarcinoma and ischemic stroke, with focus on (co-)morbidity and aging / cellular senescence (SASKit): protocol for a prospective cohort study

|                               |   |
|-------------------------------|---|
| Journal:                      | <i>BMJ Open</i>   |
| Manuscript ID                 | bmjopen-2020-039560.R1  |
| Article Type:                 | Protocol  |
| Date Submitted by the Author: | 06-Oct-2020   |
| Complete List of Authors:     | <p>Henze, Larissa; Rostock University Medical Center, Department of Hematology, Oncology and Palliative Medicine, Department of Medicine III, Research Focus Oncology, Rostock University Medical Center</p> <p>Walter, Uwe; Rostock University Medical Center, Department of Child and Adolescence Psychiatry and Neurology, Centre for Transdisciplinary Neurosciences Rostock, Rostock University Medical Center</p> <p>Murua Escobar, Hugo; Rostock University Medical Center, Department of Hematology, Oncology and Palliative Medicine, Department of Medicine III, Research Focus Oncology, Rostock University Medical Center</p> <p>Junghanß, Christian; Rostock University Medical Center, Department of Hematology, Oncology and Palliative Medicine, Department of Medicine III, Research Focus Oncology, Rostock University Medical Center</p> <p>Jaster, Robert; Rostock University Medical Center, Department of Gastroenterology, Research Focus Oncology, Rostock University Medical Center</p> <p>Köhling, Rüdiger; Rostock University Medical Center, Oscar Langendorff Institute of Physiology, Centre for Transdisciplinary Neurosciences Rostock, Rostock University Medical Center, Ageing of Individuals and Society, Interdisciplinary Faculty, Rostock University</p> <p>Lange, Falko; Rostock University Medical Center, Oscar Langendorff Institute of Physiology, Centre for Transdisciplinary Neurosciences Rostock, Rostock University Medical Center</p> <p>Salehzadeh-Yazdi, Ali; University of Rostock, Department of Systems Biology and Bioinformatics</p> <p>Wolkenhauer, Olaf; University of Rostock, Department of Systems Biology and Bioinformatics, Centre for Transdisciplinary Neurosciences Rostock, Rostock University Medical Center</p> <p>Hamed, Mohamed; Rostock University Medical Center, Institute for Biostatistics and Informatics in Medicine and Ageing Research, Research Focus Oncology, Rostock University Medical Center</p> <p>Barrantes, Israel; Rostock University Medical Center, Institute for Biostatistics and Informatics in Medicine and Ageing Research</p> <p>Palmer, Daniel; Rostock University Medical Center, Institute for Biostatistics and Informatics in Medicine and Ageing Research</p> <p>Möller, Steffen; Rostock University Medical Center, Institute for Biostatistics and Informatics in Medicine and Ageing Research</p> <p>Kowald, Axel; Rostock University Medical Center, Institute for Biostatistics and Informatics in Medicine and Ageing Research</p> |

|                                  |  |
|----------------------------------|--|
|                                  | Heussen, Nicole; RWTH Aachen University, Department of Medical Statistics, Research Focus Oncology, Rostock University Medical Center<br>Fuellen, Georg; Rostock University Medical Center, Institute for Biostatistics and Informatics in Medicine and Ageing Research, Centre for Transdisciplinary Neurosciences Rostock, Rostock University Medical Center, Research Focus Oncology, Rostock University Medical Center, Ageing of Individuals and Society, Interdisciplinary Faculty, Rostock University |
| <b>Primary Subject Heading</b> : | Diagnostics  |
| Secondary Subject Heading:       | Genetics and genomics  |
| Keywords:                        | Health informatics < BIOTECHNOLOGY & BIOINFORMATICS, Immunology < NATURAL SCIENCE DISCIPLINES, Thromboembolism < CARDIOLOGY, Molecular aspects < ONCOLOGY, Stroke < NEUROLOGY  |
|                                  |  |

SCHOLARONE™  
Manuscripts



I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our [licence](#).

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which [Creative Commons](#) licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

## Towards biomarkers for outcomes after pancreatic ductal adenocarcinoma and ischemic stroke, with focus on (co-)morbidity and aging / cellular senescence (SASKit): protocol for a prospective cohort study

Larissa Henze\*<sup>1,##</sup>, Uwe Walter\*<sup>2,#</sup>, Hugo Murua Escobar<sup>1,##</sup>, Christian Junghanß<sup>1,##</sup>, Robert Jaster<sup>3,##</sup>, Rüdiger Köhling<sup>4,#,###</sup>, Falko Lange<sup>4,#</sup>, Ali Salehzadeh-Yazdi<sup>5</sup>, Olaf Wolkenhauer<sup>5,#</sup>, Mohamed Hamed<sup>6,##</sup>, Israel Barrantes<sup>6</sup>, Daniel Palmer<sup>6</sup>, Steffen Möller<sup>6</sup>, Axel Kowald<sup>6</sup>, Nicole Heussen\*\*<sup>7</sup>, Georg Fuellen\*\*<sup>6,#,##,###</sup>

\*joint first authors

\*\*joint corresponding authors: [nheussen@ukaachen.de](mailto:nheussen@ukaachen.de), [fuellen@uni-rostock.de](mailto:fuellen@uni-rostock.de)

1 Rostock University Medical Center, Department of Hematology, Oncology and Palliative Medicine, Department of Medicine III, Rostock, Germany

2 Rostock University Medical Center, Department of Neurology, Rostock, Germany

3 Rostock University Medical Center, Department of Gastroenterology, Rostock, Germany

4 Rostock University Medical Center, Oscar Langendorff Institute of Physiology, Rostock, Germany

5 University of Rostock, Department of Systems Biology and Bioinformatics, Rostock, Germany

6 Rostock University Medical Center, Institute for Biostatistics and Informatics in Medicine and Ageing Research, Rostock, Germany

7 RWTH Aachen, Department of Medical Statistics, Aachen, Germany

# Centre for Transdisciplinary Neurosciences Rostock, Rostock University Medical Center ## Research Focus Oncology, Rostock University Medical Center ### Ageing of Individuals and Society, Interdisciplinary Faculty, Rostock University

### Abstract

**Introduction:** Aging-related processes such as cellular senescence are believed to underlie the accumulation of diseases in time, causing (co-)morbidity, including cancer, thromboembolism and stroke. Intervening into these processes may delay, stop or reverse morbidity. To study the link between (co-)morbidity and aging, by exploring biomarkers and molecular mechanisms of disease-triggered deterioration, we will recruit 50 patients with pancreatic ductal adenocarcinoma, 50 patients with (thromboembolic) ischemic stroke and 50 controls, at Rostock University Medical Center.

**Methods and Analysis:** We will gather routine blood data, clinical performance measurements and patient-reported outcomes at up to 7 points in time, and in-depth transcriptomics & proteomics at two early time points. Aiming for clinically relevant biomarkers, the primary outcome is a composite of probable sarcopenia, clinical performance (described by ECOG Performance Status for patients with pancreatic ductal adenocarcinoma and the Modified Rankin Scale for patients with stroke) and quality of life. Further outcomes cover other aspects of morbidity such as cognitive decline, and of comorbidity such as vascular or cancerous events. The data analysis is comprehensive in that it includes biostatistics & machine learning, both following standard role models & additional explorative approaches. *Predictive* biomarkers for interventions addressing senescence may become available if the biomarkers that we find are predominantly related to aging / cellular senescence. Similarly, *diagnostic* biomarkers will be explored for their relationship to aging / cellular senescence. Our findings will require validation in independent studies, and our dataset shall be useful to validate the findings of other studies. In some of the explorative analyses, we shall include insights from systems biology modeling as well as insights from preclinical animal models. We humbly suggest that our detailed study protocol and data analysis plan may also guide other biomarker exploration trials. **Ethics and Dissemination:** The study was approved by the local ethics committee, registered at the German Clinical Trials Register, and results will be published following standard guidelines.

### Article summary

Strengths and limitations of this study:

- In-depth measurements of both relevant outcomes and potential biomarkers.
- Comparatively low number of participants, for both patients and controls.
- In-depth and detailed data analysis plan.
- Investigation of the deterioration of health and (co-)morbidity, not just of survival.
- Two co-morbid diseases investigated in almost identical ways in two sub-studies.

### Introduction

**Study Rationale and Aims.** The primary aim of the SASKit (“Senescence-Associated Systems diagnostics Kit for cancer and stroke”) study is to discover a set of molecular biomarkers for outcomes after pancreatic ductal adenocarcinoma (PDAC) and ischemic stroke (IS), which are specifically useful to predict disease-triggered deterioration of health (“disease deterioration” for short) in terms of probable sarcopenia<sup>1</sup>, reduced clinical performance and quality of life (QoL). The outcomes also include the (co-)morbidity of vascular events (here defined as stroke, myocardial infarction, and venous or arterial thromboembolism) in patients with PDAC, which are observed frequently apart from sarcopenia. Also included is the (co-)morbidity of any kind of cancer and of cognitive decline following IS. Moreover, we consider mortality, as the most canonical outcome. Following up on the primary aim, we will investigate the nature of the molecular biomarkers to find out whether cellular senescence and other aging-associated processes are contributing to disease deterioration. As a secondary aim, we will search for *diagnostic* biomarkers related to cellular senescence and other aging-related processes that may differentiate healthy controls from PDAC or IS patients. Therefore, in the following we motivate our study by describing the prevalence and the outcomes of PDAC and IS, the known predictors of these outcomes, and the specific prevalence of co-morbidity and known predictors for this co-morbidity. The role of cellular senescence in aging and disease is described in Box 1. The background of the cancerous and vascular comorbidity is described in Box 2. Importantly, despite differences in disease pathology, dynamics and prognosis, there is a lot of evidence that cellular senescence is, in part, an important contributor to disease etiology, progression and consequences for both diseases. Avoiding unclear or circular terminology, we define a biomarker in a very general fashion, simply as a feature (data point)  $f_1$  that successfully predicts another feature  $f_2$  at a later time-point<sup>2</sup>, in a biomedical context. Here, features may be composite ones, based on the measurement of individual features. Often, feature  $f_1$  refers to molecular data, while feature  $f_2$  refers to phenotypic data, such as clinical outcomes. Ultimately, we aim to identify biomarkers that are easy to measure, and that are then validated in other studies to predict a clinically relevant outcome. The study design is illustrated in Figure 1, while the data analysis plan is summarized in Figure 2.

**Pancreatic ductal adenocarcinoma: prevalence and outcomes.** The incidence of pancreatic cancer is increasing; in 2017 the global incidence was 5.7 per 100,000 person-years<sup>3</sup>. Age is the most important risk factor, and incidence peaks at 65 to 69 years in males and 75 to 79 years in females<sup>3</sup>. Pancreatic ductal adenocarcinoma (PDAC) is the most common histological type of pancreatic cancer<sup>4</sup>. The disease is characterized by late clinical presentation<sup>5</sup>, early metastases and poor prognosis, with a one-year survival rate in Europe of only 15%<sup>6</sup>. Many patients have unresectable disease at the time of diagnosis, either as locally advanced disease or already with metastases. Therefore therapy is palliative consisting of chemotherapy and/or best supportive care. Disease deterioration with weight loss and low muscle strength, that is, cachexia and sarcopenia<sup>7</sup>, will follow, for some patients rapidly (within a few weeks) and for others during a longer interval of one or two years. Recent developments in oncology have not shown much benefit in clinical trials of patients with PDAC<sup>8</sup>. Inflammation, desmoplasia and early metastases are deemed responsible for the difficulties in targeting the disease.

1  
2  
3 Moreover, vascular events are frequent problems in the course of PDAC and may contribute to disease  
4 deterioration or early death. Venous thromboembolism is the most common event occurring in up to  
5 34% of patients with metastatic PDAC<sup>9,10</sup>, but arterial ischemic events, like stroke, are also reported  
6<sup>11-14 15 16</sup>, see also Box 2. Therefore, deterioration and mortality in PDAC can not only be explained by  
7 tumor progression as such, but other factors like sarcopenia/cachexia and vascular events contribute  
8 as well. Furthermore, we suggest that the underlying cause of all these factors are aging-related  
9 processes such as cellular senescence and chronic inflammation.  
10  
11

12 **Pancreatic ductal adenocarcinoma: known biomarkers and clinical scores.** In PDAC patients there is  
13 a lack of established scores describing the risk of disease deterioration and the risk of  
14 sarcopenia/cachexia in particular. Referring to the endpoint of overall survival, some recent studies  
15 tried to establish inflammation-based scores to better characterize outcome in PDAC. In a  
16 retrospective analysis of 386 patients with PDAC of different stages, CRP/Alb ratio, neutrophil-  
17 lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR) and modified Glasgow prognostic score  
18 (mGPS) were studied<sup>17</sup>. In patients with locally advanced and metastatic disease, the CRP/alb ratio  
19 was an independent factor of poor survival<sup>17</sup>. Another retrospective study evaluating CA19-9, CEA,  
20 CRP, LDH and bilirubin levels in locally advanced and metastatic pancreatic cancer patients treated  
21 with chemotherapy showed an independent prognostic significance for overall survival only for CA 19-  
22 9 decline during treatment<sup>18</sup>. Other studies have evaluated risk factors for thromboembolic events in  
23 pancreatic cancer patients and more generally in patients with cancer<sup>19</sup> (see also Box 2). The Khorana  
24 score, developed more than ten years ago, is widely used to estimate venous thromboembolic risk in  
25 the population of cancer patients<sup>20</sup>; it integrates standard laboratory parameters (platelet count,  
26 hemoglobin, leukocyte count), body mass index (BMI) and the cancer site (with pancreatic cancer and  
27 gastric cancer classified as very high risk). Still, its performance was questioned in a retrospective  
28 cohort of pancreatic cancer patients<sup>21</sup> and in a prospective cohort study of patients with different  
29 cancer types, among them 109 with pancreatic cancer<sup>19</sup>. The clinical association of PDAC,  
30 sarcopenia/cachexia and thromboembolism is well-described<sup>11</sup>, but still not understood in its  
31 pathophysiology<sup>22</sup>. Within the SASKit study we aim to identify biomarkers and molecular mechanisms  
32 contributing to this clinical association, by investigating their relation to clinically relevant outcomes.  
33  
34  
35  
36  
37  
38  
39

40 **Ischemic stroke, prevalence and outcomes.** Ischemic stroke (IS) occurs in the German population with  
41 an incidence of 236 per 100,000 per year<sup>23</sup>. The mean age of acute stroke patients is 73-74 years, with  
42 more than 80% of patients being over 60 years old. After a first stroke, nearly 5% of patients suffer a  
43 second stroke within a year. Mortality after IS is about 12% within one year and about 30% within five  
44 years<sup>23</sup>. Mild to moderately disabled stroke survivors showed an elevated prevalence of sarcopenia  
45 >6 months after onset of stroke compared with non-stroke individuals (13.2% vs 5.3%)<sup>24</sup>. The  
46 mechanisms underlying sarcopenia include loss of muscle mass, reduction of fibre cross-sectional area  
47 and increased intramuscular fat deposition occurring between 3 weeks and 6 months after stroke in  
48 both paretic and non-paretic limbs<sup>25</sup>. Comorbid, or subsequent cancer may facilitate sarcopenia after  
49 IS. A US nationwide inpatient sample study reported that 10% of hospitalized IS patients have comorbid  
50 cancer, 16% of them with gastrointestinal cancer and 1% with PDAC, and that this association may be  
51 on the rise<sup>26</sup>. Additionally, within two years after IS, another 2% to 4% of patients receive a new cancer  
52 diagnosis<sup>27-29</sup>. Within the SASKit study we aim to identify biomarkers to predict outcome after IS in  
53 terms of general health state (i.e. sarcopenia, deterioration of clinical performance, cognitive  
54 functioning, frailty) and quality of life, as well as (co-)morbidity, as we do for the PDAC cohort.  
55  
56  
57  
58  
59  
60



1  
2  
3 **Ischemic stroke, known biomarkers and clinical scores.** In an early study of 956 patients with acute IS,  
4 determinants of long-term mortality were age, obesity, cardiac arrhythmias, diabetes mellitus,  
5 coronary heart disease and organic brain syndrome at discharge from hospital; interestingly,  
6 hypercholesterolaemia and smoking did not affect long-term outcome<sup>30</sup>. More recent studies  
7 uniformly identified age and stroke severity, usually assessed on the NIHSS or similar scales, as  
8 biomarkers of long-term functional outcome and mortality after stroke<sup>31 32</sup>. Fibrinogen has been  
9 related to long-term outcome after stroke<sup>33 34</sup>. There have been conflicting data on the predictive  
10 value of serum bilirubin levels on the long term risk of cardiovascular disease. While some studies are  
11 in favor of a predictive value (e.g.:<sup>35-37</sup>), others are not (e.g.:<sup>38</sup>). Also, CRP levels have been reported  
12 to impact the functional long-term outcome after IS<sup>39</sup>, and early neurological deterioration after IS has  
13 been related to decreasing albumin levels, elevated CRP and fibrinogen levels<sup>40</sup>. Potential biomarkers  
14 for occult cancer in IS patients include elevated D-dimers, fibrinogen, and CRP; infarction in multiple  
15 vascular territories; and poor nutritional status<sup>41</sup>. Interestingly, IS patients with elevation of at least  
16 two of the following coagulation-related serum markers, that is, D-dimer, prothrombin fragment 1.2,  
17 thrombin-antithrombin complex and fibrin monomer, in the post-acute phase of stroke, were more  
18 likely to have occult cancer or recurrent stroke during follow-up for 1.4±0.8 years<sup>42</sup>. In another study  
19 of acute IS patients, high D-dimer levels at admission were independently associated with recurrent  
20 stroke and all-cause mortality during follow-up for up to 3 years<sup>43</sup>. These findings underpin the idea  
21 of shared risk factors for unfavorable outcomes in IS as well as cancer and they suggest that there may  
22 be coagulation-related biomarkers indicating an early stage of carcinogenesis or stroke (see also Box  
23 2). Nevertheless, the clinical biomarkers that currently exist for predicting outcome are limited in their  
24 performance and clinical utility, and there is a need to overcome the limitations of current predictive  
25 models<sup>44</sup>.

---

26  
27  
28  
29  
30  
31  
32 **Box 1: Aging and cellular senescence.** Extra lifetime gained over the last century led to the widespread  
33 emergence of age-related diseases that are rarely seen in younger people. Older patients are thus  
34 more likely to display several comorbidities, which makes treatment difficult and expensive. Over the  
35 last years, strong evidence has accumulated that the presence of senescent cells (i.e. non-dividing,  
36 arrested but metabolically active cells that escape apoptosis) is causally involved in diseases such as  
37 atherosclerosis, cancer, fibrosis, pancreatitis, osteoarthritis, Alzheimer disease and metabolic  
38 disorders<sup>45 46</sup>. Evidence that senescent cells are not only correlated with aging and diseases, but are  
39 instead causally involved, comes from recent studies, which transplanted senescent cells from old into  
40 young mice<sup>47</sup>. This resulted in persistent functional impairment as well as spread of cellular senescence  
41 to host tissues. Another strong line of evidence comes from experiments that actually removed  
42 senescent cells from aged mice by *senolytics*<sup>47-49</sup>. In each case an increase in lifespan and a delay of  
43 typical age related diseases was observed. Most recently, the results of human pilot trials of putative  
44 senolytic treatments in case of idiopathic pulmonary fibrosis and osteoarthritis have been reported.  
45 One team<sup>50</sup> treated idiopathic pulmonary fibrosis patients with dasatinib and quercetin and  
46 demonstrated safety as well as notable improvements in some physical abilities. Furthermore, a  
47 human phase-1 study demonstrated that a senolytic compound, which was applied locally in patients  
48 with osteoarthritis of the knee, was safe and well-tolerated<sup>51</sup>. A clinically meaningful improvement in  
49 several measures, including pain, function, as well as modulation of certain senescence-associated  
50 secretory phenotype (SASP) factors and disease-related biomarkers was observed after a single dose.

---

51  
52  
53  
54  
55  
56  
57 **Box 2: Cellular senescence and the comorbidity of cancer and vascular events.** Some cancers such as  
58 PDAC can trigger vascular events by hyper-coagulation, reflecting Trousseau's syndrome first reported  
59 150 years ago<sup>11</sup>. In turn, strong associations between coagulation, cellular senescence and the SASP  
60

1  
2  
3 were demonstrated recently <sup>52</sup>. While cellular senescence can suppress PDAC and cancerous  
4 proliferation in general, it also triggers tumor progression by fostering inflammatory processes,  
5 including the SASP, while on the other hand, after ischemic stroke, it attenuates recovery <sup>53-57</sup>. For both  
6 diseases, causal influences can be traced back to molecular determinants: PAI-1 (also known as  
7 SERPINE1 and part of the SASP) is involved in cancer-triggered thromboembolism <sup>54 56</sup> and stroke  
8 recovery in animals <sup>58</sup>. Other proteins involved in cellular senescence, specifically inflammatory  
9 cytokines such as IL6, and the lesser known osteopontin and gelsolin, are also markers for both PDAC  
10 and stroke <sup>59-62</sup>. The cyclin-dependent kinase CDK5 <sup>63</sup> is implicated in the progression of PDAC as well  
11 as in the recovery from stroke <sup>57 64</sup>. Moreover, apart from being genetic risk factors <sup>65 66</sup>, the most  
12 prominent drivers of cellular senescence (p16/CDKN2A and p21/CDKN1A) also promote PDAC  
13 progression <sup>67</sup> and endothelial embolic and arteriosclerotic mechanisms of stroke <sup>68</sup>. Finally, two small-  
14 molecule interventions into cellular senescence, fisetin and quercetin, are both potential treatments  
15 of both PDAC and stroke. In case of stroke, the blood-brain-barrier is passed by quercetin which  
16 improves stroke outcome <sup>69</sup>. In case of PDAC it was observed that quercetin inhibits pancreatic cancer  
17 growth *in-vitro* and *in-vivo* <sup>70</sup>. Fisetin is found in various fruits (especially strawberries) and it is  
18 chemically similar to quercetin, with strong putative senolytic effects, extending lifespan of mice even  
19 when intervention with fisetin started only at an advanced age <sup>71</sup>. In a study involving nude mice  
20 implanted with prostate cancer cells, treatment with fisetin significantly retarded tumor growth <sup>72</sup>.  
21 Also, in case of lung cancer, there is evidence for the beneficial effects of fisetin. One study showed  
22 that fisetin provides protection against benzo(a)pyrene [B(a)P]-induced lung carcinogenesis in albino  
23 mice <sup>73</sup> and another *in vivo* study demonstrated the synergistic effects of fisetin and cyclophosphamide  
24 in reducing the growth of lung carcinoma in mice <sup>74</sup>. Several other studies have also demonstrated its  
25 anticarcinogenic, neurotrophic and anti-inflammatory effects that are beneficial in numerous diseases,  
26 including pancreatic cancer and stroke <sup>75</sup>.  
27  
28  
29  
30  
31  
32  
33

---

## 34 Methods

35  
36  
37 The presentation is based on the reporting recommendations for tumor marker prognostic studies  
38 (REMARK), that is, items (1) – (11) of the REMARK checklist <sup>76</sup>. The study design is illustrated in Figure  
39 1, while the data analysis plan is summarized in Figure 2.  
40

### 41 Study design

42  
43 The SASKit (“Senescence-Associated Systems diagnostics Kit for cancer and stroke”) study is designed  
44 as a prospective, observational, cohort study to identify biomarkers for disease deterioration in  
45 patients with PDAC or with IS and, specifically, for the (co-)morbidity of these diseases including  
46 vascular events and sarcopenia following the diagnosis of PDAC as well as cancer and cognitive decline  
47 following IS. All patients will be treated for their diseases in accordance with current guidelines or  
48 therapy standards and at the physician's discretion. Due to the observational study design, regular  
49 treatment of the patient is not affected apart from sampling blood (20 to 80 ml at up to 7 time-points  
50 over the next years). Assessment of disease deterioration will be based on standardized clinical  
51 performance measurements, and patient reported outcomes based on questionnaires (see below for  
52 details). Additionally, data from clinical charts and information from the general practitioner will be  
53 collected. The SASKit study is divided into two subtrials with a common control group, both featuring  
54 essentially the same outcomes, predictor measurements and data analysis approaches.  
55  
56  
57

### 58 Patient and Public Involvement

59  
60 It was not possible to involve patients or the public in the design of the study.

### Characteristics of participants (patients and controls)

In the first subtrial (PDAC-subtrial), patients with an initial diagnosis of PDAC in locally advanced or metastatic stage without previous systemic therapy will be considered for enrollment, whereas patients with a (thromboembolic) IS of the supratentorial brain region within the past 3 to 10 days, with a definitive brain infarction volume >10 ml in an assessment by magnetic resonance imaging (MRI) will be considered for the second subtrial (IS-subtrial). Except for some explorative analyses, the subtrials will be analyzed separately.

Within both subtrials, eligible as controls are those without PDAC or IS and with no other malignant disease or other (hemorrhagic) stroke during the past two years. Potential controls will be recruited from persons who have lived in the same household as the patient within the last 2 years, have a maximum age difference of 12 years and are neither brothers nor sisters (i.e. spouses, second-degree relatives or friends). The controls are selected so that the age and gender structure approximately reflects the age and gender distribution of the patients. Therefore, the age and gender of the patients will be continuously recorded, and the controls selected in such a way that their frequency distribution of gender at any time corresponds approximately to that of the currently recruited patients.

The following criteria lead to exclusion from participation in the study for both patients and controls, *at time of recruitment*:

- previous or current medical tumor therapy
- other cancer within the past 2 years
- previous stroke with persistent deficit
- myocardial infarction within the past 2 years
- therapeutic anticoagulation within the past 2 years for longer than 1 month
- pre-existing dementia
- chronic heart failure stage NYHA IV
- terminal renal insufficiency with hemodialysis
- known HIV infection
- known active hepatitis C
- pregnancy
- age < 18 years.

Both subtrials will be implemented according to the same standardized protocol. After written informed consent of each participant, patients and controls will be followed up at 3, 12, 24, 36 and 48 months after their inclusion in the trial, whenever possible. The PDAC-subtrial includes an additional time-point for examinations at 6 months after inclusion, given that mortality due to PDAC is expected to be accelerated as compared to IS.

The study is expected to start in the second quarter of 2020 and will finish with the last participant's follow up at 48 months. Until that time, we expect that 50 PDAC patients, 50 IS patients, and 50 controls participated in the trial. The study will be conducted at the Rostock University Medical Center (UMR), Germany at Clinic III - Hematology, Oncology, Palliative Medicine and at the Department of Neurology; the institutions of the other co-authors are supporting the study in a variety of ways. The

1  
2  
3 study protocol has been approved by the ethics committee of the UMR. The study is registered at  
4 German Clinical Trials Register (DRKS00021184) and will be conducted following ICH-GCP.  
5

#### 6 General health- and disease-related and demographic data 7

8 General data of the study participants will be recorded at the beginning of the study (“month 0”) and  
9 consist of the following: age, sex, BMI, temperature, blood pressure, heart rate (ECG). Furthermore,  
10 through interviews the following additional data will be recorded: vascular risk factors (arterial  
11 hypertension, diabetes, hyperlipidaemia, smoking habits), history of vascular events (stroke,  
12 myocardial infarction, venous or arterial thromboembolism), atrial fibrillation, history of cancer,  
13 current medication, surgery or blood transfusions in the past three months and vascular or cancerous  
14 events affecting any first degree relatives. These data may provide influential factors for explorative  
15 analyses, or be employed to interpret and discuss the results of the study.  
16  
17

#### 18 Blood sampling 19

20 Blood sampling will be done in a standardized fashion, that is, fasting and between 8 and 10 am, for all  
21 assays. Routine blood parameters will be recorded at the time-points described above (months 0 to  
22 48). These consist of differential blood count, INR (International normalized ratio of prothrombin time),  
23 partial thromboplastin time, D-dimers, fibrinogen, factor XII, albumin, bilirubin, high-sensitive CRP,  
24 CA19-9, cholesterol, and HbA1c. Among the standard measurements, we also measure the liver  
25 parameters ALT, AST and AP as surrogate markers of liver disease.  
26  
27

28 Experimental blood analysis (PAI-1 and omics) will be done for patients at month 0 in case of PDAC, at  
29 month 0 or at month 3 in case of stroke (where the 3-month time point is taken if it reflects a better  
30 state of the patient as described by the NIHSS), and furthermore at month 3 in case of PDAC, and at  
31 month 12 in case of stroke. For controls, the experimental blood analysis will be carried out at month  
32 0 and at month 12, assuming that for these, data do not change much in the 3 months after baseline.  
33 The justification for taking the better state in case of stroke is the maximization of differences with the  
34 12 months follow-up data. In terms of practicality (being able to calculate a biomarker signature  
35 sooner), however, the state at month 0 should be selected for all stroke patients. Since the blood  
36 sample will be taken pre-processed and frozen at month 0 in all cases, we are in principle able to  
37 perform the experimental blood analysis for all stroke patients at month 0, and we can do this analysis  
38 in retrospect if deemed necessary. We also take blood of PDAC patients at month 12, to have the  
39 option to do an experimental blood analysis if deemed useful. In the following we will refer to the  
40 *baseline* time-point (month 0, or month 3 in cases of stroke patients that improved) and the *landmark*  
41 time-point (month 3 for PDAC patients and month 12 for stroke patients and controls). The  
42 experimental blood analysis is done earlier for PDAC because of high expected mortality within the  
43 first year.  
44  
45  
46  
47

48 The experimental blood analysis includes PAI-1 (see *Box 2*) as well as high-throughput (omics) analyses,  
49 that is, transcriptomics and proteomics analysis in T-cells and proteomics of serum. T cells are of  
50 interest because these were reported to carry the strongest signal with respect to cellular senescence,  
51 based on the marker p16<sup>77</sup>. We intend to measure gelsolin and osteopontin as well, provided that  
52 sufficiently standardized assays become available in due time; the blood collected for this  
53 measurement shall otherwise be used to measure cytokines/chemokines such as IL6, IL8 and TNF $\alpha$ ,  
54 which are part of the SASP, by ELISA assays. At time of writing, we do not yet have reliable estimates  
55 on the amount of blood cells still available for measuring protein expression, so an antibody-based  
56 protein array (in case of low amounts), or mass spectrometry (in case of sufficiently high amounts) will  
57 be used alternatively. For the blood serum, we intend to use the same protein measurement method.  
58 In the default case of a protein array, we plan to use the novel but dedicated “Senescence Associated  
59  
60

1  
2  
3 Secretory Phenotype (SASP) Antibody Sampler Kit” (consisting of approx. 10 SASP-related proteins  
4 being measured; Cell Signaling Technology) for both cellular and serum proteomics. Further  
5 exploratory molecular analyses not (yet) funded but permitted based on the ethics approval include  
6 the following: single-cell analyses of blood, methylation assays for calculating epigenetic clocks <sup>78</sup>,  
7 genetics by SNP array or whole-genome sequencing, and telomere length. A separate ethics approval  
8 was granted for an optional skin biopsy; skin microbiome analyses are planned as well. More  
9 specifically, participants have the option to provide a skin biopsy of 5 mm from an area that is not  
10 usually visible. We expect that about 30-50% of the participants will opt in. We keep the biopsy in  
11 culture for several days and divide it into several pieces. Using these, we measure biomarkers of  
12 cellular senescence (specifically, senescence-associated beta-galactosidase, which cannot easily be  
13 measured in blood) and we treat some pieces with compounds that may affect cellular senescence,  
14 such as quercetin or fisetin. Moreover, we plan to sample the microbiome of the forehead using a  
15 standard swab. This is a very simple procedure, motivated by the claim that a competitive epigenetic  
16 aging clock can be based on such a sample <sup>79</sup>.

20  
21 Blood sample processing for the experimental analysis will be performed according to standard  
22 operating procedures (SOP) at the research laboratory of Clinic III - Hematology, Oncology, Palliative  
23 Medicine. The procedures include flow cytometric control of the sampling quality including distribution  
24 of cell types and vitality as performed in routine diagnostics. Isolation of peripheral blood mononuclear  
25 cells (PBMCs) will also be performed following the SOP used by the laboratory in routine diagnostics.  
26 T-Cell separation will be performed according to an established work flow based on magnetic bead  
27 purification via Miltenyi MACS following manufacturer’s instructions. T cell fraction purity as well as  
28 vitality will then be verified by flow cytometric analyses as described above. Nucleic acid isolation as  
29 well as protein isolation will be further performed according to the SOP of the research laboratory  
30 performed using column separation (Qiagen, Hilden Germany). RNA integrity values (RIN) will be  
31 analysed using an Agilent Scientific Instruments Bioanalyzer as instructed by the manufacturer. RIN  
32 values above 6 will qualify for RNAseq or Clariom D Array analyses; for RNAseq average reads per  
33 sample will be set at approx. 40 x 10e6.

### 37 Clinical performance measurements and patient-reported outcomes

39 At baseline and at each follow-up, handgrip strength (“grip strength” for short) is measured using a  
40 digital hand dynamometer (Jamar Plus). The test is performed while sitting comfortably, shoulder  
41 adducted, elbow placed on the tabletop and flexed to 90 degrees, with the forearm and wrist in a  
42 neutral position <sup>80</sup>. The highest value of three measurements of maximal isometric contraction of the  
43 dominant hand, or if paralyzed due to IS, contraction of the unaffected hand, is documented in kg.  
44 Further, the following clinical performance measurements are evaluated by the study physician or  
45 study nurse according to standard protocols: ECOG Performance Status (ECOG PS) <sup>81</sup>, modified Rankin  
46 Scale (mRS) <sup>82</sup>, Canadian Study on Health & Aging Clinical Frailty Scale (CSHA-CFS) <sup>83</sup>, NIH-Stroke Scale  
47 (NIHSS) <sup>84</sup>, Montreal Cognitive Assessment (MOCA) <sup>85</sup>. All raters are certified for the applicable scores  
48 (mRS, NIHSS, MOCA). Patient-reported outcomes (measured by questionnaires) are the following: EQ-  
49 5D-5L and EQ-VAS (generic evaluation of QoL in 5 domains and overall on a visual analog scale) <sup>86</sup>,  
50 HADS-D (evaluation of anxiety and depression) <sup>87</sup>, WHODAS 2.0 (WHO Disability Assessment Schedule)  
51 <sup>88</sup>, and, for patients with PDAC, FACIT-Pal (evaluating QoL with focus on palliative symptoms and needs)  
52 <sup>89, 90</sup>. All questionnaires are administered following the suppliers’ instructions.

### 56 Follow up data

58 Apart from the clinical and patient-reported outcomes, further follow-up data are BMI, temperature,  
59 blood pressure, heart rate (ECG), atrial fibrillation, current medication, tumor treatment, comorbidity  
60 (any vascular or cancer event), hospital admissions or palliative care. Additionally, based on clinical



charts and information from the general practitioner, we will record medication, (co-)morbidity and mortality. Just like the general health- and disease-related and demographic data recorded at time of recruitment, these data may provide influential factors for explorative analyses, or be employed to interpret and discuss the results of the study.

## Endpoints

In both subtrials, the primary endpoint is a composite measure of “disease deterioration” defined as the *first* occurrence within a follow-up interval of at least one of the following.

- a. Sarcopenia, measured by grip strength less than 27 kg for males and less than 16 kg for females (according to the revised European consensus, EWGSOP2, <sup>1</sup>).
- b. Deterioration of clinical performance, that is, of the ECOG PS by at least two points (PDAC-subtrial), or of the mRS by at least one point (IS-subtrial).
- c. Deterioration of QoL, described as a reduction of the EQ-5D-5L by at least 0.07 in the index score, **and** deterioration of at least 7 points in the EQ-VAS (ranging from 0-100).

Deterioration will be considered between baseline (month 0) and the respective follow-up investigation. As described above, for patients with IS who have improved their condition (measured by NIHSS) within the first 3 months, this time point (month 3) will be used as a baseline instead. Item (a) is the deterioration from “no sarcopenia” to “probable sarcopenia” as defined by current consensus <sup>1</sup>. Grip strength has been widely used for assessing muscle strength, which is currently used as the most reliable measure of muscle function, loss of which indicating sarcopenia <sup>1</sup>. ECOG PS is established in describing the general condition of patients with cancer, whereas mRS is established in patients with stroke. Death is reflected by both scores as ECOG PS of 5 or mRS of 6, and it will always consider death from any cause. The EQ-5D-5L evaluates QoL in five dimensions (mobility, self-care, usual activity, pain/discomfort, and anxiety/depression), all relevant for patients with PDAC and IS. Furthermore, it is a generic score so that results will be comparable for different diseases (as recently described in patients with stroke <sup>91</sup>) and for the general population <sup>92</sup>). Even though disease-specific scores might evaluate symptom burden in even more detail, the EQ-5D-5L was recently shown to be comparable to QoL scores developed specifically for pulmonary embolism and deep vein thrombosis (that is, PEmb-QoL, VEINES-QOL/Sym and PACT-Q2) in terms of acceptability, validity and responsiveness <sup>93</sup>. A clinical deterioration in EQ-5D-5L is described as a minimal important difference in the range from 0.07 to 0.09 index points and in VAS from 7 to 10 <sup>94</sup> which is the basis for the definition of item (c). Controls reach their endpoint by the same definition as the subcohort for which they serve as control; in any integrative analysis of both subtrials, a deterioration of the mRS by at least one point will be used as the criterion (instead of ECOG PS), because stroke patients in general have a slower deterioration than PDAC patients, and controls naturally have the slowest expected deterioration.

The primary composite endpoint and all secondary endpoints will be evaluated in a first analysis, based on data obtained until summer 2021, and in a second analysis, based on data obtained until summer 2023, and in a third analysis at the end of the study. The second analysis may be delayed until data of 90% of the study participants are available (at least including the month 12 follow up) and it may then constitute the “main” analysis of the study.

The following secondary endpoints are evaluated:

- each component of the primary endpoint (separately);
- occurrence of disease-specific (co-)morbidity, as follows
  - new vascular events (stroke, myocardial infarction, venous or arterial thromboembolism), specifically in patients with PDAC;



- new cancer, specifically in patients with IS;
- probable sarcopenia (based on grip strength);
- cognitive decline (deterioration of MOCA by 3 points from best value at baseline);
- frailty, defined as a CSHA-CFS level of 6, 7, or 8;
- all-cause mortality.

Further, a sum-score summarizing all measurements of phenotypic variables (grip strength, clinical performance measurements, comorbid events, mortality) will be considered as a surrogate for “aging”, normalizing all continuous-scaled components in order to obtain a common scale with an average of zero and standard deviation of one. The components of the sum-score will all be given equal weight.

### Predictors

While all phenotypic features (grip strength, clinical performance, patient reported outcomes, comorbid events, mortality) are contributing to the definition of endpoints (as dependent variables/parameters), all routine and experimental blood features (PAI-1, omics) are considered to be potential predictors; these are also called the independent variables/parameters. This delineation is justified by (a) the paradigm that (clinical) relevance is tied to high-level phenotypes describing health and survival, specifically including QoL <sup>2</sup>, and (b) the goal of developing a “senescence-associated systems diagnostics kit” that includes a careful selection of biomarkers contributing, as much as possible, also to molecular-mechanistic insights into PDAC, IS and their (co-)morbidity, which we hypothesize to be related to cellular senescence and aging. Age and gender will be included as mandatory covariates (also termed confounders, that is, predictors which we do not aim to explore, or which we wish to improve upon) in all statistical models. Further covariates are smoking, liver dysfunction or disease, the baseline NIHSS score in case of IS, as well as locally-advanced vs metastatic PDAC and modality of treatment in case of PDAC. As described, the successful predictors identified by our study, following the statistical analyses outlined below, are called biomarkers; we wish to stress that these are only *candidates* for the ultimate goal of *clinically validated biomarkers*; in particular, they still need to be validated in further studies (based, e.g., on other cohorts). A set of biomarkers is also called a biomarker signature.

### Blinding and pseudonymization

No blinding will be done during the study. However, the primary composite endpoint will be documented without subjective influence due to standardized definitions. Thus, detection bias will be kept at a minimal extent. Furthermore, information bias will be minimized as we will use simple measurements, which are applied in daily practice or are self-reported and easy to perform (e.g. EQ-5D-5L). The rigorous inclusion of all eligible patients within the recruitment period will help to minimize selection bias. All patient data are pseudonymized to all investigators except for the attending physician and study nurse. Since all major data analyses are based on known information about the outcomes (e.g., supervised machine learning with cross-validation), the data analysis will also be performed based on the pseudonymized data. Protection of personal and clinical data of all patients and controls will follow all relevant legal regulations.

### Sample size

No formal sample size calculation was performed a-priori for this observational study. The prevalence of PDAC combined with the requirement to complete the study within a reasonable timeframe implied a target of 50 patients per group (PDAC, IS and control group). Nevertheless, a power analysis revealed that a sample size of 50 patients will have 80% power to detect a significant difference by a non-

1  
2  
3 parametric Wilcoxon statistic between an AUC of 0.75 for a particular biomarker signature compared  
4 to the null hypothesis value of 0.5 at a significance level of 5% under the assumption that about three  
5 times as many patients will reach the primary endpoint, compared to patients who will not reach the  
6 primary endpoint <sup>95</sup>.  
7

## 8 **Data Analysis Plan**

### 9

10 **General considerations:** The guiding criteria for biomarker identification in the SASKit study are the  
11 maximization of the predictive signal, clinical relevance/utility, biomedical/molecular/clinical  
12 interpretability, and practicality/cost. Given the relatively low number of participants in this in-depth  
13 study, to maximize the signal for the endpoints and predictors given as outlined above, we must aim  
14 to use all available information. Regarding endpoints, whenever possible, we thus wish to consider the  
15 (censored) time-to-event information inherent in the baseline and follow-up examinations, and in the  
16 mortality data. The primary endpoint was defined to integrate expected clinical utility and maximum  
17 signal. In defining the (secondary) endpoints, we considered an array of clinically relevant single  
18 endpoints as well as a sum-score of all phenotypic measurements; we hypothesize that the latter  
19 carries the largest amount of signal. Given the small sample, we cannot set aside an extra validation  
20 dataset. For the predictors considered to be covariates/confounders, please see the section on  
21 “Predictors”, above. The data analysis plan is summarized in Figure 2.  
22  
23  
24  
25

26  
27 **Data quality assessment and cleaning:** The need for (and the amount of) data cleaning cannot easily  
28 be estimated beforehand; we plan to follow the MarkAGE guidelines <sup>96</sup> to deal with missing values,  
29 and to detect and rectify outliers and batch artefacts.  
30

31 **Predictor/Feature integration:** Regarding predictors (features), we first need to remember that we  
32 measure at baseline (at months 0 or 3) and at one landmark (main follow-up, that is, at months 3 or  
33 12). While use of baseline features is unrestricted, use of landmark features is, of course, restricted to  
34 predict outcomes after the landmark. Further, we need to handle the high dimensionality of the omics  
35 features. Here, upfront feature integration, e.g., by averaging measurements as described below, is  
36 considered preferable specifically for the high-dimensional omics data, for the following reasons.  
37  
38  
39

- 40 1) A small feature space allows for an easier understanding and interpretation, see, e.g., <sup>97</sup>.
- 41 2) Integrated features can be used as input for both the standard biostatistics and the standard  
42 machine learning parts of the analysis.
- 43 3) Use of few features is more time-tested than newer methods featuring the joint calculation of  
44 the prediction model and the selection of the features, albeit the latter are quite often claimed  
45 to be superior by their developers.
- 46 4) Naturally, feature integration avoids multicollinearity and overfitting, and multiple testing is  
47 less of an issue. This counters the “curse of dimensionality” and “de-noises” the data towards  
48 better prediction performance <sup>97 98</sup>.
- 49 5) Feature integration allows the handling of feature heterogeneity, which in our case refers to  
50 routine blood measurements as well as various omics data types.
- 51 6) In the *explorative* analyses, systems biology modelling and the parallelogram approach are  
52 both supposed to deliver further small sets of integrated, highly informative features, which  
53 may, e.g., dominate systems behaviour, or which are believed to translate well from animal  
54 models to humans (see below).  
55  
56  
57  
58  
59  
60

1  
2  
3 While most features will be available for the baseline and the landmark time-point, utilizing baseline  
4 data is clinically more useful, simply because the prediction for the endpoint is available much earlier.  
5 Nevertheless, in the explorative analyses, we will investigate the predictive power of *changes* in  
6 feature measurements from baseline to landmark, given that such changes may be more informative  
7 about future disease deterioration (and other endpoints) than just baseline values.  
8  
9

10  
11 **Specific omics data feature integration:** Notably, we face a heterogeneous “multi-view” dataset,  
12 usually referred to as “multi-omics”. Our feature integration approach (see above) is also known as a  
13 “late integration” type of analysis, implying that measurements for different omics data types are  
14 reduced early on to activation scores for pathways or subnetworks that are then integrated at a “late”  
15 level. To calculate the activation scores for subnetworks, we use, by default, the  
16 ExprEssence/FocusHeuristics *linkscore*<sup>99 100</sup>, taking the links (gene/protein interactions) from a  
17 functional interaction network defaulting to STRING. Our experience with the *linkscore* motivates us  
18 to include this method as one of the approaches proposed for feature integration in the following,  
19 influencing the calculation of up to 10 features on which the standard biostatistics and machine  
20 learning shall be based. Specifically, we take the average expression measurement for all patients  
21 (as a list of expression values, one per gene) and the average for all controls (as a list of expression  
22 values, one per gene) to calculate a *linkscore* for each STRING interaction, and assemble a  
23 “condensed” network including all interactions with a *linkscore* in that percentile for which the 50  
24 highest-scoring interactions are shown. These interactions form subnetworks. We then take the  
25 average *linkscore* for each subnetwork as the subnetwork activation score. Alternative methods  
26 such as *keypathwayminer* will be used in the exploratory analyses, see below. For the pathways (such  
27 as KEGG), we will calculate pathway activation scores using Gene Set Variation Analysis (GSVA)<sup>101</sup>. This  
28 method calculates pathway activation scores from expression data, is suited for use with microarray  
29 as well as RNAseq data and performed strongly in a recent benchmarking analysis<sup>102</sup>. The GSVA-based  
30 pathway activation scores can subsequently be compared between patients and controls in the same  
31 way as normal gene expression data, calculating, for each pathway, a fold-change of the pathway  
32 activation scores between patients and controls. Here, we average over all patients and over all  
33 controls, respectively, using the *limma* R package and adjusting for age and gender of the individual  
34 patient/control pathway activation. An example of this approach is given in the GSVA publication,  
35 where differential pathway activation was identified between acute lymphoblastic lymphoma and  
36 mixed-lineage lymphoma<sup>101</sup>. The major downside of feature integration may be information loss;  
37 subsequent statistical and machine-learning-based analyses receive only a tiny fraction of the amount  
38 of information that is available in total.  
39

40 Gene expression data (transcriptomics) will be our preferred omics data type. Nevertheless, proteins  
41 are closer to the phenotype than transcripts, so we wish to not ignore these. Therefore, we prepare to  
42 deal with both kinds of proteome data that we may expect (see “Experimental blood analyses”, above),  
43 as follows.  
44

- 45 1. Large-scale data, likely based on mass spectrometry, in the order of hundreds or more proteins  
46 that can be identified and measured in all the conditions investigated differentially.
- 47 2. Small-scale data, likely based on antibody arrays, in the order of tens or less.

48 Except for the raw data preprocessing depending on the platform, once log-fold changes describing  
49 differential expression are established, we thus expect to handle the large-scale proteome data  
50 essentially the same as the transcriptomics data, and the small-scale proteome data similarly to the  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

blood routine data, for cells and serum alike. Overall, the omics data are expected to come along three main coordinates, that is,

1. as blood cell transcriptomics and proteomics as well as serum proteomics;
2. longitudinal in time (for baseline and landmark); and
3. for PDAC, IS and control.

All coordinates can be exploited for differential analyses, even though the PDAC and IS data will be analyzed separately except for some integrative *explorative* analyses (see below). In the *explorative* analyses, the *longitudinal* transcriptomics of the patients and controls will also be analyzed together, see below. For the standard biostatistics and machine learning analyses, we plan to employ 5 approaches to feature integration, each yielding a shortlist of 5 integrated features, as follows.

- 1) **(5 features)** A first shortlist of features will consist of the following expert selection from the routine blood measurements (incl. PAI-1): *neutrophil-lymphocyte-ratio*, *fibrinogen*, *high-sensitive C-reactive protein*, *albumin* and *PAI-1*.
- 2) **(5 features)** For the cellular gene expression measurements, we use ExprEssence/FocusHeuristics (see above) to calculate *the top-5 subnetworks scoring highest*.
- 3) **(5 features)** Again for the cellular gene expression measurements, we use GSVA (see above) to calculate the top-5 most strongly changing pathways as features.
- 4) + 5) **(10 features)**
  - a) In case of dealing with large-scale serum proteomics data, we proceed as in (2) + (3);
  - b) In case of dealing with small-scale serum proteomics data, we proceed as follows:
    - i) if the number of features measured successfully is in the order of 10, we refrain from any processing;
    - ii) if the number of features is in the order of around 10-100, we select the 10 features with the smallest p-values indicating differences between the mean values of patient and control, based on a t-test.

For genomic features as per (2), the feature measurements for an individual patient or control will then be the average linkscores of the 5 selected subnetworks, contrasting each patient with average control data, and each control with average patient data. For genomic features as per (3), the feature measurements for each patient/control will be the GSVA scores of the 5 selected pathways. By construction, we expect the resulting features to reflect the up/downregulation of disease-related transcripts/proteins or pathways/subnetworks. Using the GSVA-based integrated features as input to the biostatistical analyses employing Cox proportional hazard models, we are in fact closely following the “Survival analysis in ovarian carcinoma” example as described in the GSVA publication <sup>101</sup>. Regarding the expert selection from the routine blood measurements, we are aware that some of these features may be considered to have an almost trivial relationship to outcome prediction for the diseases we study; e.g. fibrinogen may correlate strongly with the size of the stroke-damaged brain area and may thus be considered a covariate. However, to our knowledge, none of these features are validated clinical biomarkers, and it is quite possible that a combination of simple biomarkers is key to the best possible prediction. We selected the *neutrophil-lymphocyte-ratio* specifically because it is cheap to measure; it is, however, like many other blood-based features, easily influenced by acute infection.

**Exploratory feature integration:** Apart from the FocusHeuristics/ExprEssence *linkscore*, we employ alternatives such as *keypathwayminer* <sup>103</sup>. Further, we calculate pathway activation scores for the

1  
2  
3 following senescence-related KEGG pathways, which include PAI-1 (see the Introduction) but do not  
4 refer to a specific disease, as of February 2020: *Cellular senescence*, *HIF-1 signaling pathway*, *p53*  
5 *signaling pathway*, *Apelin signaling pathway*, *Hippo signaling pathway*, *Complement and coagulation*  
6 *cascades*. “Early integration” by, e.g., first averaging transcript and protein expression on a single-gene  
7 basis, is also planned.  
8  
9

10  
11 **Choice of data analysis methods for biomarker discovery:** We will consider two main approaches of  
12 data analysis, one motivated by statistical methods, the other by machine learning approaches. While  
13 this delineation may ultimately be meaningless, we consider that regression is the core ingredient of  
14 the former, while supervised learning characterizes the latter. We will apply “standard” methods  
15 (mostly in biostatistics) and explore novel approaches (mostly in machine learning; preserving signal  
16 implies a focus on *supervised* approaches in this case). Data analysis for biomarker *discovery* trials in a  
17 *clinical* setting is usually described with a biostatisticians’ mindset, who also developed methods to  
18 cope with the high dimensionality of omics data (see below). On the other hand, the challenges of  
19 omics data also spurred the recent publication of many methods adopting machine learning, which  
20 however did not yet make it into clinical trial analysis routine, but which we wish to test (see below).  
21 We will focus on methods readily available in SAS or as R packages. Notably, the correct choice of  
22 method depends in part on known unknowns such as the strength of the signal (incl. the amount of  
23 missing data) in the routine blood measurements and the omics.  
24  
25  
26  
27  
28

29  
30 **Prediction model quality measures:** Unlike intervention trials with their highly standardized aim of  
31 establishing a statistically significant superiority (or non-inferiority) of one intervention compared to  
32 another (or to standard of care), observational biomarker trials are a more recent development with  
33 fewer precisely quantified criteria of success, and a stronger need to consider the effect size: even if a  
34 biomarker signature enables a significant improvement in predicting an outcome, raising the accuracy  
35 of the prediction, say, from 70% to 75% may not be clinically meaningful, depending on prevalence of  
36 the condition to be predicted, the cost of the biomarker measurement, etc. We thus aim to identify  
37 biomarkers making a maximum of *difference* in prediction accuracy, if we are able to compare to  
38 established scores (see also below). For the biostatistics part, the concordance statistics (c-index) will  
39 be used as an overall measure of predictive accuracy, and time-dependent ROC curves and AUC will  
40 be used to summarize the predictive accuracy at different cut-off points in time. For the machine  
41 learning part, the cross-validated accuracy and AUC/c-index, following<sup>97</sup>, are used, and to take care of  
42 a potential Simpson’s paradox we will either analyse the data stratified by gender, or we will add such  
43 an analysis and check for consistency. More generally, to investigate the role of confounders (and, if  
44 necessary, to correct for these) in the machine learning part, we wish to use the permutation technique  
45 described<sup>104</sup>. We expect that we can identify a set of biomarkers that affords an accuracy of 75% or  
46 more or an AUC of 0.75 or more in correctly predicting the primary endpoint with a precision of +/-  
47 12%<sup>105</sup>. This estimate of precision is based on half the width of a 95% confidence interval (CI) for a  
48 probability of 75%, by extension of item 6 of the tables of Sorzano et al<sup>105</sup>, which shows precision up  
49 to a sample size of N=30.  
50  
51  
52  
53  
54  
55

56  
57 **Standard biostatistical analyses:** A Cox proportional hazards regression model adjusted for age and  
58 gender will be used to estimate the hazard ratio (HR) and corresponding 95% CI to predict the primary  
59 composite endpoint separately within the PDAC cohort and IS cohort. The 5 shortlists of 5 features  
60 (see above) will be providing the canonical predictors, analyzed together. For selection of the most  
important features that might be related to the primary endpoint we will use a procedure proposed



1  
2  
3 by Sauerbrei et al.<sup>106</sup>, as follows. First, 100 bootstrap samples will be generated. Then, a multivariate  
4 Cox proportional hazards regression model with backward elimination with selection level of 0.05 will  
5 be fitted to each replication of the original data set. In a second step features with a relative selection  
6 frequency of 30% or less over all bootstrap samples will be eliminated. In a third step each feature  $X_i$   
7 for which the hypothesis of independence in combination with a feature  $X_j$  can be rejected will be  
8 eliminated if  $X_i$  is less important when  $X_j$  is included in the model, or if it does not gain importance  
9 when  $X_j$  is excluded from the model. All remaining features will be included in the final model.  
10 Graphical and numerical methods will be performed to establish the validity of the proportionality  
11 assumption<sup>107</sup> in the final model. Results will be reported as p-values, HRs and corresponding 95%-CIs.  
12 A p-value of  $p \leq 0.05$  will be interpreted as indicating statistical significance. From the final model a risk  
13 score will be calculated by multiplying the individual feature measurement of a patient with the  
14 estimated regression coefficient of each feature. The c-index will be used as an overall measure of  
15 predictive accuracy of the resulting score, a time-dependent ROC curve and AUC will be used to  
16 summarize the predictive accuracy of the score at specific times. All secondary endpoints will be  
17 evaluated using the same approach as for the primary endpoint except for the sum-score used as a  
18 surrogate for “aging”. For this endpoint, a linear mixed effects model with random intercept and spatial  
19 power covariance structure will be fitted to the data to estimate the progression of “aging”. The  
20 covariance structure is chosen to reflect the unequal intervals of follow up investigations. Model  
21 assumptions and model fit will be checked by visual inspection of residuals, and influence diagnostics.  
22 Missing values will be taken into account by a likelihood-based approach within the framework of  
23 mixed linear models with the assumption that missing values occur at random. Results will be reported  
24 as p-value assessed at a level of significance of 5% accompanied by the value of the test statistic and  
25 degrees of freedom. In addition, 95% CIs for the progression (slope) will be provided.

26  
27  
28  
29  
30  
31  
32  
33  
34 **Additional exploratory biostatistical analyses:** Again, the primary composite endpoint as well as all  
35 secondary endpoints will be evaluated separately within the PDAC cohort and IS cohort of the  
36 respective sub-trials. In a first approach, univariate Cox proportional hazard models adjusted for age  
37 and gender will be calculated for each omics feature (R package *survival*) using a cut-off of 0.05 on the  
38 false discovery rate. In a second approach, all omics features will be simultaneously considered in a  
39 multivariate Cox model, adjusted for age and gender. Towards this aim, a component-wise likelihood-  
40 based boosting algorithm proposed by Binder and Schumacher 2008<sup>108</sup> (R package *CoxBoost*) will be  
41 used to develop a biomarker signature.

42  
43  
44  
45 **Standard machine learning:** For the machine learning part, the primary outcome and all secondary  
46 outcomes give rise to an assignment of predictor/feature lists to survival times, one such list per study  
47 participant, for which biomarkers are then learned in a supervised fashion. As described, in the  
48 standard analyses, feature integration (see above) will precede the actual calculation of the model  
49 (“deep” learning approaches that take in “all” features are part of the *exploratory* analyses, see below).  
50 In the same way as the standard biostatistics analyses, the same 5 shortlists of 5 features each (see  
51 above) will be providing the canonical predictors, analyzed together. Exploiting time-to-event  
52 information, we will employ random survival forests (RSF) as described by<sup>109</sup> with the following  
53 advantages.

- 54 1. RSF can now be considered a time-tested approach, and it was the subject of a recent  
55 extensive review<sup>67</sup> and of a systematic comparison with LASSO approaches in the case without  
56  
57  
58  
59  
60



1  
2  
3 feature selection (see item 7 of the tables of Pi *et al*<sup>110</sup>, for its competitive performance which  
4 is not reflected in their abstract).

- 5  
6 2. RSF can also work on essentially all features, without a preceding feature integration/selection  
7 step, and then be compared, in the explorative machine learning analyses described below, to  
8 survival support vector machines (SSVM) and to a novel method Path2Surv that “conjointly”  
9 performs feature selection and model training, see<sup>97</sup>.
- 10  
11 3. RSF was recently compared to Cox-nnet<sup>111</sup>, a neural network approach which we consider as  
12 very promising for the *exploratory* part, see also below.
- 13  
14 4. RSF offers a considerable degree of interpretability, given that RSFs are derived from decision  
15 trees.
- 16  
17 5. RSF is considered “completely data driven and thus independent of model assumptions” and  
18 “in case of high dimensional data, limitations of univariate regression approaches such as  
19 overfitting, unreliable estimation of regression coefficients, inflated standard errors or  
20 convergence problems do not apply”<sup>67</sup>.

21  
22 In the machine learning part, we calculate accuracy and AUC/c-index using cross-validation to make  
23 the best use of our limited sample size, following the setup of<sup>97</sup> and<sup>110</sup> (who, however, set aside  
24 separate validation datasets), and we assess the features as biomarkers by ranking them by their  
25 variable importance score.

26  
27  
28 **Additional exploratory machine learning:** Apart from the more time-tested standard machine learning  
29 described above, we will also explore methods that were proposed recently, for which it is less  
30 straightforward to tell whether these methods are fit-for-purpose in our case, even though they are  
31 usually claimed to be superior by their developers based on some test/validation data sets. Specifically,  
32 as mentioned above, we expect to test Path2Surv and SSVM<sup>97</sup> as well as Cox-nnet<sup>111</sup> (without prior  
33 feature integration); the latter in particular promises a high degree of interpretability. We further  
34 explore CNet (employing the censored-data variant), for interpretable biomarkers. We also plan to  
35 employ the PASNet<sup>112</sup>, SurvivalNet<sup>113</sup> and SVRc<sup>72</sup> packages. The longitudinal transcriptomics of the  
36 patients and the controls may also be analyzed integratively based on the “optimal discovery  
37 procedure”<sup>114</sup>, considering, however, that landmark feature data can only be used to predict events  
38 after the landmark. Finally, we will map the differential omics data onto a human “healthspan pathway  
39 map”<sup>115</sup>, that is, a set of clusters/pathways based on health-related genetic data that we assembled  
40 recently.

41  
42  
43 **Explorative systems biology modelling, explorative parallelogram approach and transfer learning:**

44  
45  
46 As mentioned, systems biology modelling and parallelogram<sup>116 117</sup> extrapolation are supposed to  
47 deliver small sets of highly informative features, by contributing features that are dominating model  
48 behaviour or that are shown to translate from the SASKIt animal model data. Given the comparatively  
49 small number of study participants (but in-depth measurements), we also wish to explore “transfer  
50 learning”, which aims to utilize large amounts of public knowledge in the form of latent variables.  
51 Specifically, we plan to use, and wish to develop further, the Multiplier<sup>118</sup> approach motivated by the  
52 analysis of rare-disease data. Multiplier utilizes the RNASeq-based recount2 compendium, and apart  
53 from the functional network and pathway data that we use in the feature selection part, this  
54 compendium is expected to be our main source of biological knowledge that enters the calculations  
55 for biomarker discovery.

1  
2  
3 **Miscellaneous exploratory approaches and discovery of diagnostic biomarkers:** We will also use  
4 unsupervised machine learning to generate descriptive multi-omics correlation networks, as they were  
5 most recently employed by <sup>119</sup>, there supplemented by linear mixed effects models using (un-  
6 )restricted maximum likelihood approaches; in this very recent biomarker discovery trial of similar  
7 design as ours, but with many more longitudinal omics measurement time-points than ours, we could  
8 not identify other biomarker discovery methods being used. If genetic data become available, we will  
9 include these in some analyses; specifically, we will investigate the added value of *expression*  
10 *quantitative trait loci* (eQTL) analyses. PDAC and IS data will be analyzed together in some integrative  
11 *exploratory* analyses. In that case, the occurrence of specific endpoints will be evaluated according to  
12 the group membership (PDAC or IS). This means that in addition to the biomarker signature, a group  
13 variable, indicating PDAC or IS patients, will be included in the analysis, to assess the difference in the  
14 progression of the respective endpoints between PDAC and IS patients. We also wish to compare PDAC  
15 and IS patient data to data of healthy controls (adjusted for age and gender) by means of logistic  
16 regression models with the aim of identifying candidate biomarkers for the diagnosis of the respective  
17 disease; we then specifically investigate the association of these diagnostic biomarker candidates with  
18 cellular senescence and other aging-related processes (see also the next paragraph).  
19  
20  
21  
22  
23  
24

25 **Further analyses, and comparison with existing biomarkers and biomarker signatures:** Towards the  
26 end, we will investigate the overlap for the various biomarker identification approaches we employed,  
27 assuming that the most frequently found biomarkers may be the most robust and valid ones.  
28 Moreover, we will compare with existing biomarkers and signatures. Regarding the prediction of  
29 vascular events, we will specifically calculate the Khorana and related scores <sup>19</sup> for comparison, and  
30 report the difference in performance. Further, for all biomarkers we find, we will check their  
31 association with cellular senescence, by manual inspection, literature investigation, comparison to  
32 CellAge <sup>120</sup> and the SASP Atlas <sup>52</sup> or by formal enrichment analyses if the number of biomarkers is  
33 sufficiently large to do this in a meaningful way. Also, in a final step, we plan to identify and filter out  
34 the biomarkers that are volatile in the controls. In addition, a comparison of the biomarker profiles  
35 before and after the co-morbid event is aimed for. Finally, for publicly available data of other trials  
36 with a sufficient overlap with our predictors, we will use these as validation datasets.  
37  
38  
39  
40  
41

## 42 Discussion

### 43 Limitations

44  
45 Arguably, the most serious limitation of the SASKit study is the low number of participants. We  
46 mentioned above that in the 4-year-time-frame of the entire study, at the Rostock University Medical  
47 Center we cannot expect to recruit many more than the 50 PDAC patients to be included in this study;  
48 we could recruit more stroke patients and more controls, but given the call for proposals that allowed  
49 this exploratory (not confirmatory) study to be applied for and funded, we considered that within a  
50 limited budget, in-depth omics characterization, animal models (to be detailed in a follow up  
51 publication) and a comprehensive data analysis plan including systems biology modelling were  
52 important aspects of our study that we did not want to exclude.  
53  
54  
55

56 The two most obvious risks to the main goal of finding good biomarkers for the primary outcome based  
57 on the standard data analysis are the following. First, we found it hard to estimate the distribution of  
58 events as defined by the primary outcome; we cannot exclude that too many events take place already  
59 at the start of the study, or until the first follow-up, specifically in the PDAC subtrial, limiting the  
60 amount of information available to the subsequent time-to-event analyses. Then again, had we

1  
2  
3 defined the primary outcome more conservatively, there would have been a chance that not enough  
4 events happen until the end of the study. Second, we could not identify role-model publications  
5 reporting results of biomarker explorations that made use of machine learning methods, except for,  
6 to some extent,<sup>119</sup>, so that we enter unknown territory to some degree. The two most obvious risks  
7 to our goal of investigating the role of cellular senescence in the (co-)morbidity of PDAC and IS could  
8 be an insufficient prevalence of co-morbid events, and the complex role of treatment in case of PDAC,  
9 where additional cellular senescence is most likely triggered by therapeutic intervention<sup>121</sup>. Then  
10 again, all molecular high-throughput analyses are essentially explorative and we are open to  
11 discovering biomarkers of disease that do *not* relate to any of our pre-specified hypotheses.  
12

### 13 Implications

14  
15  
16 We designed the SASKit study to synergistically deliver upon a couple of aims that we consider to be  
17 of relevance for specific disease prognosis and treatment as well as for primary, secondary and tertiary  
18 prevention. Employing clinical performance measurements and patient-reported outcomes, we aim  
19 for clinical relevance and we suggest that prognostic biomarker signatures for general health and QoL  
20 are perhaps more important than (progression-free) survival, although there is much more data about  
21 the latter than the former. Moreover, good disease treatment options are still lacking for PDAC as well  
22 as for stroke, and the more we find cellular senescence implicated in disease deterioration, at least in  
23 a subgroup of patients with a specific biomarker signature, the more confidently we can suggest, and  
24 further explore, seno-therapeutic interventions for these two diseases.  
25

26  
27  
28 Notably, we are in the process of starting a parallel human study testing, in healthy elderly people,  
29 interventions into cellular senescence, based on *food* rich in seno-interventional compounds, and we  
30 expect that many aspects of the study design presented herein will be adopted in that parallel study.  
31 That study will also investigate aging- and senescence-related outcomes, and as such it can be seen as  
32 a test of a cautious yet potentially very effective approach to primary prevention; if the *diagnostic*  
33 biomarkers we find in the SASKit study relate to cellular senescence, this observation would constitute  
34 further evidence for (cautious) seno-interventions, moving towards a kind of universal approach of  
35 disease prevention by tackling fundamental aging-related processes (see Boxes 1 and 2).  
36

37  
38  
39 Secondary prevention, aiming to reduce the impact of a disease that has already occurred, can  
40 ultimately be supported by the SASKit study, if we can demonstrate, and (in follow up studies) confirm,  
41 a distinctive role of cellular senescence (and/or other aging-related processes such as  
42 inflammation/inflammaging<sup>122</sup>) in disease deterioration as defined here. Finally, evidence for tertiary  
43 prevention by seno-therapeutic intervention, aiming to attenuate the impact of an ongoing disease, is  
44 also an option based on how accurate, relevant and specific our biomarkers will be.  
45

46  
47  
48 Last but not least, we expect that the in-depth molecular analyses that we wish to conduct will provide  
49 mechanistic insights into the etiology of the diseases we study here, which we just see as models for  
50 the investigation of the fundamental role of aging in general and cellular senescence in particular in  
51 disease and dysfunction.

### 52 Abbreviations:

|    |        |                            |
|----|--------|----------------------------|
| 53 | ALT    | Alanine Aminotransferase   |
| 54 | AP     | Alkaline Phosphatase       |
| 55 | AST    | Aspartate Aminotransferase |
| 56 | AUC    | Area Under the Curve       |
| 57 | BMI    | Body Mass Index            |
| 58 | CA19-9 | Carbohydrate Antigen       |
| 59 | CEA    | Carcinoembryonic antigen   |
| 60 |        |                            |

|    |        |   |
|----|--------|---|
| 1  |        |   |
| 2  |        |   |
| 3  | CI     | Confidence interval   |
| 4  | CRP    | C-reactive protein  |
| 5  | ECOG   | Eastern Cooperative Oncology Group                                  |
| 6  | HR     | Hazard ratio  |
| 7  | INR    | International normalized ratio                                      |
| 8  | IS     | Ischemic Stroke   |
| 9  | LDH    | Lactate dehydrogenase   |
| 10 | LDH    | Lactate dehydrogenase   |
| 11 | NIHSS  | NIH-Stroke Scale  |
| 12 | NIHSS  | NIH-Stroke Scale  |
| 13 | NYHA   | New York Heart Association  |
| 14 | PDAC   | Pancreatic Ductal Adenocarcinoma                                    |
| 15 | PS     | Performance status  |
| 16 | QoL    | Quality of Life   |
| 17 | QoL    | Quality of Life   |
| 18 | ROC    | Receiver-Operator Characteristic                                    |
| 19 | RSF    | Random survival forests   |
| 20 | SASKit | Senescence-Associated Systems diagnostics Kit for cancer and stroke |
| 21 | SASP   | Senescence Associated Secretory Phenotype                           |
| 22 |        |   |
| 23 |        |   |
| 24 |        |   |
| 25 |        |   |
| 26 |        |   |
| 27 |        |   |
| 28 |        |   |
| 29 |        |   |
| 30 |        |   |
| 31 |        |   |
| 32 |        |   |
| 33 |        |   |
| 34 |        |   |
| 35 |        |   |
| 36 |        |   |
| 37 |        |   |
| 38 |        |   |
| 39 |        |   |
| 40 |        |   |
| 41 |        |   |
| 42 |        |   |
| 43 |        |   |
| 44 |        |   |
| 45 |        |   |
| 46 |        |   |
| 47 |        |   |
| 48 |        |   |
| 49 |        |   |
| 50 |        |   |
| 51 |        |   |
| 52 |        |   |
| 53 |        |   |
| 54 |        |   |
| 55 |        |   |
| 56 |        |   |
| 57 |        |   |
| 58 |        |   |
| 59 |        |   |
| 60 |        |   |

### Contributorship statement

Conception, writing and revision: Larissa Henze, Uwe Walter, Hugo Murua Escobar, Christian Junghanß, Robert Jaster, Rüdiger Köhling, Falko Lange, Ali Salehzadeh-Yazdi, Olaf Wolkenhauer, Mohamed Hamed, Israel Barrantes, Daniel Palmer, Steffen Möller, Axel Kowald, Nicole Heussen, Georg Fuellen.

Specific clinical considerations: Larissa Henze, Uwe Walter.

Specific experimental considerations: Hugo Murua Escobar.

Data analysis plan: Daniel Palmer, Nicole Heussen, Georg Fuellen.

Acquisition of funding: Larissa Henze, Uwe Walter, Hugo Murua Escobar, Christian Junghanß, Robert Jaster, Rüdiger Köhling, Ali Salehzadeh-Yazdi, Olaf Wolkenhauer, Georg Fuellen.

Project coordination: Axel Kowald, Georg Fuellen.

### Conflict of Interest

Dr. Walter reports personal fees from Ipsen Pharma, grants and personal fees from Merz Pharma, personal fees from Allergan, personal fees from Bristol-Myers Squibb, personal fees from Daiichi Sankyo, personal fees from Bayer Vital, personal fees from Boehringer Ingelheim, personal fees from Pfizer, personal fees from Thieme, and personal fees from Elsevier Press, all outside the submitted work. The other authors have nothing to disclose.

### Funding

We acknowledge the financial support by the Federal Ministry of Education and Research (BMBF) of Germany for the SASKit study (FKZ 01ZX1903A). The funder had no role in the design of the study.

### Data sharing statement

No data available.

### References

1. Cruz-Jentoft AJ, Bahat G, Bauer J, et al. Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing* 2019;48(1):16-31. doi: 10.1093/ageing/afy169 [published Online First: 2018/10/13]
2. Fuellen G, Jansen L, Cohen AA, et al. Health and Aging: Unifying Concepts, Scores, Biomarkers and Pathways. *Aging and Disease* 2019;10(4):883-900.
3. Collaborators GBDPC. The global, regional, and national burden of pancreatic cancer and its attributable risk factors in 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Gastroenterol Hepatol* 2019;4(12):934-47. doi: 10.1016/S2468-1253(19)30347-4
4. Kleeff J, Korc M, Apte M, et al. Pancreatic cancer. *Nat Rev Dis Primers* 2016;2:16022. doi: 10.1038/nrdp.2016.22 [published Online First: 2016/05/10]
5. Llop E, P EG, Duran A, et al. Glycoprotein biomarkers for the detection of pancreatic ductal adenocarcinoma. *World J Gastroenterol* 2018;24(24):2537-54. doi: 10.3748/wjg.v24.i24.2537 [published Online First: 2018/07/03]
6. Carrato A, Falcone A, Ducreux M, et al. A Systematic Review of the Burden of Pancreatic Cancer in Europe: Real-World Impact on Survival, Quality of Life and Costs. *J Gastrointest Cancer* 2015;46(3):201-11. doi: 10.1007/s12029-015-9724-1 [published Online First: 2015/05/15]
7. Cruz-Jentoft AJ, Sayer AA. Sarcopenia. *Lancet* 2019;393(10191):2636-46. doi: 10.1016/S0140-6736(19)31138-9 [published Online First: 2019/06/07]
8. Taieb J, Pointet AL, Van Laethem JL, et al. What treatment in 2017 for inoperable pancreatic cancers? *Ann Oncol* 2017;28(7):1473-83. doi: 10.1093/annonc/mdx174 [published Online First: 2017/05/02]
9. Menapace LA, Peterson DR, Berry A, et al. Symptomatic and incidental thromboembolism are both associated with mortality in pancreatic cancer. *Thromb Haemost* 2011;106(2):371-8. doi: 10.1160/TH10-12-0789 [published Online First: 2011/06/30]
10. Grilz E, Posch F, Konigsbrugge O, et al. Association of Platelet-to-Lymphocyte Ratio and Neutrophil-to-Lymphocyte Ratio with the Risk of Thromboembolism and Mortality in Patients with Cancer. *Thromb Haemost* 2018;118(11):1875-84. doi: 10.1055/s-0038-1673401 [published Online First: 2018/10/09]
11. Bonnerot M, Humbertjean L, Mione G, et al. Cerebral ischemic events in patients with pancreatic cancer: A retrospective cohort study of 17 patients and a literature review. *Medicine (Baltimore)* 2016;95(26):e4009. doi: 10.1097/MD.0000000000004009 [published Online First: 2016/07/02]
12. Navi BB, Reiner AS, Kamel H, et al. Association between incident cancer and subsequent stroke. *Ann Neurol* 2015;77(2):291-300. doi: 10.1002/ana.24325 [published Online First: 2014/12/05]
13. Varki A. Trousseau's syndrome: multiple definitions and multiple mechanisms. *Blood* 2007;110(6):1723-9. doi: 10.1182/blood-2006-10-053736 [published Online First: 2007/05/15]
14. Grilz E, Marosi C, Konigsbrugge O, et al. Association of complete blood count parameters, d-dimer, and soluble P-selectin with risk of arterial thromboembolism in patients with cancer. *J Thromb Haemost* 2019;17(8):1335-44. doi: 10.1111/jth.14484 [published Online First: 2019/05/18]
15. Poiree S, Monnier-Cholley L, Tubiana JM, et al. Acute abdominal aortic thrombosis in cancer patients. *Abdom Imaging* 2004;29(4):511-3. doi: 10.1007/s00261-003-0144-5 [published Online First: 2004/03/17]
16. Schattner A, Klepfish A, Huszar M, et al. Two patients with arterial thromboembolism among 311 patients with adenocarcinoma of the pancreas. *Am J Med Sci* 2002;324(6):335-8. doi: 10.1097/00000441-200212000-00009 [published Online First: 2002/12/24]
17. Liu Z, Jin K, Guo M, et al. Prognostic Value of the CRP/Alb Ratio, a Novel Inflammation-Based Score in Pancreatic Cancer. *Ann Surg Oncol* 2017;24(2):561-68. doi: 10.1245/s10434-016-5579-3 [published Online First: 2016/09/22]
18. Haas M, Heinemann V, Kullmann F, et al. Prognostic value of CA 19-9, CEA, CRP, LDH and bilirubin levels in locally advanced and metastatic pancreatic cancer: results from a multicenter, pooled



- 1  
2  
3 analysis of patients receiving palliative chemotherapy. *J Cancer Res Clin Oncol*  
4 2013;139(4):681-9. doi: 10.1007/s00432-012-1371-3
- 5  
6 19. van Es N, Di Nisio M, Cesarman G, et al. Comparison of risk prediction scores for venous  
7 thromboembolism in cancer patients: a prospective cohort study. *Haematologica*  
8 2017;102(9):1494-501. doi: 10.3324/haematol.2017.169060 [published Online First:  
9 2017/05/28]
- 10  
11 20. Khorana AA, Kuderer NM, Culakova E, et al. Development and validation of a predictive model for  
12 chemotherapy-associated thrombosis. *Blood* 2008;111(10):4902-7. doi: 10.1182/blood-2007-  
13 10-116327 [published Online First: 2008/01/25]
- 14  
15 21. Kruger S, Haas M, Burkl C, et al. Incidence, outcome and risk stratification tools for venous  
16 thromboembolism in advanced pancreatic cancer - A retrospective cohort study. *Thromb Res*  
17 2017;157:9-15. doi: 10.1016/j.thromres.2017.06.021 [published Online First: 2017/07/05]
- 18  
19 22. Faille D, Bourrienne MC, de Raucourt E, et al. Biomarkers for the risk of thrombosis in pancreatic  
20 adenocarcinoma are related to cancer process. *Oncotarget* 2018;9(41):26453-65. doi:  
21 10.18632/oncotarget.25458 [published Online First: 2018/06/15]
- 22  
23 23. Stahmeyer J, Stubenrauch S, Geyer S, et al. The frequency and timing of recurrent stroke—an  
24 analysis of routine health insurance data. *Dtsch Arztebl Int* 2019;116:711-7.
- 25  
26 24. Ryan AS, Ivey FM, Serra MC, et al. Sarcopenia and Physical Function in Middle-Aged and Older  
27 Stroke Survivors. *Arch Phys Med Rehabil* 2017;98(3):495-99. doi: 10.1016/j.apmr.2016.07.015  
28 [published Online First: 2016/08/18]
- 29  
30 25. Scherbakov N, von Haehling S, Anker SD, et al. Stroke induced Sarcopenia: muscle wasting and  
31 disability after stroke. *Int J Cardiol* 2013;170(2):89-94. doi: 10.1016/j.ijcard.2013.10.031  
32 [published Online First: 2013/11/16]
- 33  
34 26. Sanossian N, Djabiras C, Mack WJ, et al. Trends in cancer diagnoses among inpatients hospitalized  
35 with stroke. *J Stroke Cerebrovasc Dis* 2013;22(7):1146-50. doi:  
36 10.1016/j.jstrokecerebrovasdis.2012.11.016 [published Online First: 2012/12/19]
- 37  
38 27. Uemura J, Kimura K, Sibazaki K, et al. Acute stroke patients have occult malignancy more often than  
39 expected. *Eur Neurol* 2010;64(3):140-4. doi: 10.1159/000316764 [published Online First:  
40 2010/07/30]
- 41  
42 28. Cocho D, Gendre J, Boltès A, et al. Predictors of occult cancer in acute ischemic stroke patients. *J*  
43 *Stroke Cerebrovasc Dis* 2015;24(6):1324-8. doi: 10.1016/j.jstrokecerebrovasdis.2015.02.006  
44 [published Online First: 2015/04/18]
- 45  
46 29. Selvik HA, Thomassen L, Bjerkreim AT, et al. Cancer-Associated Stroke: The Bergen NORSTROKE  
47 Study. *Cerebrovasc Dis Extra* 2015;5(3):107-13. doi: 10.1159/000440730 [published Online  
48 First: 2015/12/10]
- 49  
50 30. Weitbrecht WU, Kirchhoff D. [Long-term prognosis of cerebral infarct in comparison with a normal  
51 population]. *Versicherungsmedizin* 1995;47(2):46-9. [published Online First: 1995/04/01]
- 52  
53 31. Meyer S, Verheyden G, Brinkmann N, et al. Functional and motor outcome 5 years after stroke is  
54 equivalent to outcome at 2 months: follow-up of the collaborative evaluation of rehabilitation  
55 in stroke across Europe. *Stroke* 2015;46(6):1613-9. doi: 10.1161/STROKEAHA.115.009421  
56 [published Online First: 2015/05/09]
- 57  
58 32. Drozdowska BA, Singh S, Quinn TJ. Thinking About the Future: A Review of Prognostic Scales Used  
59 in Acute Stroke. *Front Neurol* 2019;10:274. doi: 10.3389/fneur.2019.00274 [published Online  
60 First: 2019/04/06]
33. Pedersen A, Stanne TM, Redfors P, et al. Fibrinogen concentrations predict long-term cognitive  
outcome in young ischemic stroke patients. *Res Pract Thromb Haemost* 2018;2(2):339-46. doi:  
10.1002/rth2.12078 [published Online First: 2018/07/27]
34. Swarowska M, Polczak A, Pera J, et al. Hyperfibrinogenemia predicts long-term risk of death after  
ischemic stroke. *J Thromb Thrombolysis* 2014;38(4):517-21. doi: 10.1007/s11239-014-1122-1  
[published Online First: 2014/08/12]



- 1
  - 2
  - 3
  - 4
  - 5
  - 6
  - 7
  - 8
  - 9
  - 10
  - 11
  - 12
  - 13
  - 14
  - 15
  - 16
  - 17
  - 18
  - 19
  - 20
  - 21
  - 22
  - 23
  - 24
  - 25
  - 26
  - 27
  - 28
  - 29
  - 30
  - 31
  - 32
  - 33
  - 34
  - 35
  - 36
  - 37
  - 38
  - 39
  - 40
  - 41
  - 42
  - 43
  - 44
  - 45
  - 46
  - 47
  - 48
  - 49
  - 50
  - 51
  - 52
  - 53
  - 54
  - 55
  - 56
  - 57
  - 58
  - 59
  - 60
35. Perlstein TS, Pande RL, Creager MA, et al. Serum total bilirubin level, prevalent stroke, and stroke outcomes: NHANES 1999-2004. *Am J Med* 2008;121(9):781-88 e1. doi: 10.1016/j.amjmed.2008.03.045 [published Online First: 2008/08/30]
36. Choi Y, Lee SJ, Spiller W, et al. Causal Associations Between Serum Bilirubin Levels and Decreased Stroke Risk: A Two-Sample Mendelian Randomization Study. *Arterioscler Thromb Vasc Biol* 2020;40(2):437-45. doi: 10.1161/ATVBAHA.119.313055 [published Online First: 2019/12/06]
37. Zhong P, Wu D, Ye X, et al. Association of circulating total bilirubin level with ischemic stroke: a systemic review and meta-analysis of observational evidence. *Ann Transl Med* 2019;7(14):335. doi: 10.21037/atm.2019.06.71 [published Online First: 2019/09/03]
38. Jorgensen ME, Torp-Pedersen C, Finer N, et al. Association between serum bilirubin and cardiovascular disease in an overweight high risk population from the SCOUT trial. *Nutr Metab Cardiovasc Dis* 2014;24(6):656-62. doi: 10.1016/j.numecd.2013.12.009 [published Online First: 2014/02/19]
39. Wang L, Li Y, Wang C, et al. C-reactive Protein, Infection, and Outcome After Acute Ischemic Stroke: A Registry and Systematic Review. *Curr Neurovasc Res* 2019;16(5):405-15. doi: 10.2174/1567202616666191026122011 [published Online First: 2019/11/19]
40. Martin AJ, Price CI. A Systematic Review and Meta-Analysis of Molecular Biomarkers Associated with Early Neurological Deterioration Following Acute Stroke. *Cerebrovasc Dis* 2018;46(5-6):230-41. doi: 10.1159/000495572 [published Online First: 2018/12/06]
41. Navi BB, Iadecola C. Ischemic stroke in cancer patients: A review of an underappreciated pathology. *Ann Neurol* 2018;83(5):873-83. doi: 10.1002/ana.25227 [published Online First: 2018/04/11]
42. Ellis D, Rangaraju S, Duncan A, et al. Coagulation markers and echocardiography predict atrial fibrillation, malignancy or recurrent stroke after cryptogenic stroke. *Medicine (Baltimore)* 2018;97(51):e13830. doi: 10.1097/MD.00000000000013830 [published Online First: 2018/12/24]
43. Nezu T, Kitano T, Kubo S, et al. Impact of D-dimer levels for short-term or long-term outcomes in cryptogenic stroke patients. *J Neurol* 2018;265(3):628-36. doi: 10.1007/s00415-018-8742-x [published Online First: 2018/01/27]
44. Chaudhary D, Abedi V, Li J, et al. Clinical Risk Score for Predicting Recurrence Following a Cerebral Ischemic Event. *Front Neurol* 2019;10:1106. doi: 10.3389/fneur.2019.01106 [published Online First: 2019/11/30]
45. Yanai H, Fraifeld VE. The role of cellular senescence in aging through the prism of Koch-like criteria. *Ageing Res Rev* 2018;41:18-33. doi: 10.1016/j.arr.2017.10.004 [published Online First: 2017/11/07]
46. Gonzalez-Meljem JM, Apps JR, Fraser HC, et al. Paracrine roles of cellular senescence in promoting tumourigenesis. *Br J Cancer* 2018;118(10):1283-88. doi: 10.1038/s41416-018-0066-1 [published Online First: 2018/04/20]
47. Xu M, Pirtskhalava T, Farr JN, et al. Senolytics improve physical function and increase lifespan in old age. *Nat Med* 2018;24(8):1246-56. doi: 10.1038/s41591-018-0092-9 [published Online First: 2018/07/11]
48. Baker DJ, Childs BG, Durik M, et al. Naturally occurring p16(Ink4a)-positive cells shorten healthy lifespan. *Nature* 2016;530(7589):184-9. doi: 10.1038/nature16932 [published Online First: 2016/02/04]
49. Baar MP, Brandt RMC, Putavet DA, et al. Targeted Apoptosis of Senescent Cells Restores Tissue Homeostasis in Response to Chemotoxicity and Aging. *Cell* 2017;169(1):132-47 e16. doi: 10.1016/j.cell.2017.02.031 [published Online First: 2017/03/25]
50. Justice JN, Nambiar AM, Tchkonja T, et al. Senolytics in idiopathic pulmonary fibrosis: Results from a first-in-human, open-label, pilot study. *EBioMedicine* 2019 doi: 10.1016/j.ebiom.2018.12.052
51. UNITY. UNITY Biotechnology Reports Promising Topline Data from Phase 1 First-in-human Study of UBX0101 in Patients with Osteoarthritis of the Knee, 2019.

- 1  
2  
3 52. Tanaka T, Biancotto A, Moaddel R, et al. Plasma proteomic signature of age in healthy humans. *Aging Cell* 2018;17(5):e12799. doi: 10.1111/accel.12799 [published Online First: 2018/07/12]
- 4  
5 53. Childs BG, Gluscevic M, Baker DJ, et al. Senescent cells: an emerging target for diseases of ageing. *Nat Rev Drug Discov* 2017;16(10):718-35. doi: 10.1038/nrd.2017.116 [published Online First: 2017/07/22]
- 6  
7  
8  
9 54. Hisada Y, Mackman N. Cancer-associated pathways and biomarkers of venous thrombosis. *Blood* 2017;130(13):1499-506. doi: 10.1182/blood-2017-03-743211 [published Online First: 2017/08/16]
- 10  
11  
12 55. Moir JA, White SA, Mann J. Arrested development and the great escape--the role of cellular senescence in pancreatic cancer. *Int J Biochem Cell Biol* 2014;57:142-8. doi: 10.1016/j.biocel.2014.10.018 [published Online First: 2014/12/03]
- 13  
14  
15 56. Valenzuela CA, Quintanilla R, Moore-Carrasco R, et al. The Potential Role of Senescence As a Modulator of Platelets and Tumorigenesis. *Front Oncol* 2017;7:188. doi: 10.3389/fonc.2017.00188 [published Online First: 2017/09/13]
- 16  
17  
18 57. Posada-Duque RA, Barreto GE, Cardona-Gomez GP. Protection after stroke: cellular effectors of neurovascular unit integrity. *Front Cell Neurosci* 2014;8:231. doi: 10.3389/fncel.2014.00231 [published Online First: 2014/09/02]
- 19  
20  
21 58. Chan SL, Bishop N, Li Z, et al. Inhibition of PAI (Plasminogen Activator Inhibitor)-1 Improves Brain Collateral Perfusion and Injury After Acute Ischemic Stroke in Aged Hypertensive Rats. *Stroke* 2018;49(8):1969-76. doi: 10.1161/STROKEAHA.118.022056 [published Online First: 2018/07/12]
- 22  
23  
24 59. Garcia-Berrocoso T, Penalba A, Boada C, et al. From brain to blood: New biomarkers for ischemic stroke prognosis. *J Proteomics* 2013;94:138-48. doi: 10.1016/j.jprot.2013.09.005 [published Online First: 2013/09/26]
- 25  
26  
27 60. Mendioroz M, Fernandez-Cadenas I, Rosell A, et al. Osteopontin predicts long-term functional outcome among ischemic stroke patients. *J Neurol* 2011;258(3):486-93. doi: 10.1007/s00415-010-5785-z [published Online First: 2010/10/23]
- 28  
29  
30 61. Pan S, Chen R, Brand RE, et al. Multiplex targeted proteomic assay for biomarker detection in plasma: a pancreatic cancer biomarker case study. *J Proteome Res* 2012;11(3):1937-48. doi: 10.1021/pr201117w [published Online First: 2012/02/10]
- 31  
32  
33 62. Poruk KE, Firpo MA, Scaife CL, et al. Serum osteopontin and tissue inhibitor of metalloproteinase 1 as diagnostic and prognostic biomarkers for pancreatic adenocarcinoma. *Pancreas* 2013;42(2):193-7. doi: 10.1097/MPA.0b013e31825e354d [published Online First: 2013/02/15]
- 34  
35  
36 63. Alexander K, Yang HS, Hinds PW. Cellular senescence requires CDK5 repression of Rac1 activity. *Mol Cell Biol* 2004;24(7):2808-19. doi: 10.1128/mcb.24.7.2808-2819.2004 [published Online First: 2004/03/17]
- 37  
38  
39 64. Feldmann G, Mishra A, Hong SM, et al. Inhibiting the cyclin-dependent kinase CDK5 blocks pancreatic cancer formation and progression through the suppression of Ras-Ral signaling. *Cancer Res* 2010;70(11):4460-9. doi: 10.1158/0008-5472.CAN-09-1107 [published Online First: 2010/05/21]
- 40  
41  
42 65. Akinyemi R, Tiwari HK, Arnett DK, et al. APOL1, CDKN2A/CDKN2B, and HDAC9 polymorphisms and small vessel ischemic stroke. *Acta Neurol Scand* 2018;137(1):133-41. doi: 10.1111/ane.12847 [published Online First: 2017/10/05]
- 43  
44  
45 66. Cremin C, Howard S, Le L, et al. CDKN2A founder mutation in pancreatic ductal adenocarcinoma patients without cutaneous features of Familial Atypical Multiple Mole Melanoma (FAMMM) syndrome. *Hered Cancer Clin Pract* 2018;16:7. doi: 10.1186/s13053-018-0088-y [published Online First: 2018/03/16]
- 46  
47  
48 67. Wang T, Notta F, Navab R, et al. Senescent Carcinoma-Associated Fibroblasts Upregulate IL8 to Enhance Prometastatic Phenotypes. *Mol Cancer Res* 2017;15(1):3-14. doi: 10.1158/1541-7786.MCR-16-0192 [published Online First: 2016/09/30]
- 49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 68. Chen J, Huang X, Halicka D, et al. Contribution of p16INK4a and p21CIP1 pathways to induction of  
4 premature senescence of human endothelial cells: permissive role of p53. *Am J Physiol Heart*  
5 *Circ Physiol* 2006;290(4):H1575-86. doi: 10.1152/ajpheart.00364.2005 [published Online First:  
6 2005/10/26]  
7  
8 69. Tressera-Rimbau A, Arranz S, Eder M, et al. Dietary Polyphenols in the Prevention of Stroke.  
9 *Oxidative medicine and cellular longevity* 2017;2017:7467962. doi: 10.1155/2017/7467962  
10  
11 70. Angst E, Park JL, Moro A, et al. The flavonoid quercetin inhibits pancreatic cancer growth in vitro  
12 and in vivo. *Pancreas* 2013;42(2):223-9. doi: 10.1097/MPA.0b013e318264ccae  
13  
14 71. Yousefzadeh MJ, Zhu Y, McGowan SJ, et al. Fisetin is a senotherapeutic that extends health and  
15 lifespan. *EBioMedicine* 2018;36:18-28. doi: 10.1016/j.ebiom.2018.09.015  
16  
17 72. Khan FM, Zubek VB. Support Vector Regression for Censored Data (SVRc): A Novel Tool for Survival  
18 Analysis. Eighth IEEE International Conference on Data Mining. Pisa, Italy, 2008.  
19  
20 73. Ravichandran N, Suresh G, Ramesh B, et al. Fisetin, a novel flavonol attenuates benzo(a)pyrene-  
21 induced lung carcinogenesis in Swiss albino mice. *Food and chemical toxicology : an*  
22 *international journal published for the British Industrial Biological Research Association*  
23 2011;49(5):1141-7. doi: 10.1016/j.fct.2011.02.005  
24  
25 74. Touil YS, Seguin J, Scherman D, et al. Improved antiangiogenic and antitumour activity of the  
26 combination of the natural flavonoid fisetin and cyclophosphamide in Lewis lung carcinoma-  
27 bearing mice. *Cancer Chemother Pharmacol* 2011;68(2):445-55. doi: 10.1007/s00280-010-  
28 1505-8  
29  
30 75. Khan N, Syed DN, Ahmad N, et al. Fisetin: a dietary antioxidant for health promotion. *Antioxid*  
31 *Redox Signal* 2013;19(2):151-62. doi: 10.1089/ars.2012.4901 [published Online First:  
32 2012/11/06]  
33  
34 76. Altman DG, McShane LM, Sauerbrei W, et al. Reporting Recommendations for Tumor Marker  
35 Prognostic Studies (REMARK): explanation and elaboration. *PLoS Med* 2012;9(5):e1001216.  
36 doi: 10.1371/journal.pmed.1001216 [published Online First: 2012/06/08]  
37  
38 77. Liu Y, Sanoff HK, Cho H, et al. Expression of p16(INK4a) in peripheral blood T-cells is a biomarker of  
39 human aging. *Aging Cell* 2009;8(4):439-48. doi: 10.1111/j.1474-9726.2009.00489.x  
40  
41 78. Ward-Caviness CK, Huffman JE, Everett K, et al. DNA methylation age is associated with an altered  
42 hemostatic profile in a multiethnic meta-analysis. *Blood* 2018;132(17):1842-50. doi:  
43 10.1182/blood-2018-02-831347  
44  
45 79. Huang S, Haiminen N, Carrieri AP, et al. Human Skin, Oral, and Gut Microbiomes Predict  
46 Chronological Age. *mSystems* 2020;5(1) doi: 10.1128/mSystems.00630-19 [published Online  
47 First: 2020/02/13]  
48  
49 80. Sousa-Santos AR, Amaral TF. Differences in handgrip strength protocols to identify sarcopenia and  
50 frailty - a systematic review. *BMC Geriatr* 2017;17(1):238. doi: 10.1186/s12877-017-0625-y  
51 [published Online First: 2017/10/19]  
52  
53 81. Oken MM, Creech RH, Tormey DC, et al. Toxicity and response criteria of the Eastern Cooperative  
54 Oncology Group. *Am J Clin Oncol* 1982;5(6):649-55. [published Online First: 1982/12/01]  
55  
56 82. van Swieten JC, Koudstaal PJ, Visser MC, et al. Interobserver agreement for the assessment of  
57 handicap in stroke patients. *Stroke* 1988;19(5):604-7. doi: 10.1161/01.str.19.5.604 [published  
58 Online First: 1988/05/01]  
59  
60 83. Rockwood K, Song X, MacKnight C, et al. A global clinical measure of fitness and frailty in elderly  
61 people. *CMAJ* 2005;173(5):489-95. doi: 10.1503/cmaj.050051 [published Online First:  
62 2005/09/01]  
63  
64 84. Lyden P, Brott T, Tilley B, et al. Improved reliability of the NIH Stroke Scale using video training.  
65 NINDS TPA Stroke Study Group. *Stroke* 1994;25(11):2220-6. doi: 10.1161/01.str.25.11.2220  
66 [published Online First: 1994/11/01]  
67  
68 85. Nasreddine ZS, Phillips NA, Bedirian V, et al. The Montreal Cognitive Assessment, MoCA: a brief  
69 screening tool for mild cognitive impairment. *J Am Geriatr Soc* 2005;53(4):695-9. doi:  
70 10.1111/j.1532-5415.2005.53221.x [published Online First: 2005/04/09]

- 1
- 2
- 3
- 4 86. Herdman M, Gudex C, Lloyd A, et al. Development and preliminary testing of the new five-level
- 5 version of EQ-5D (EQ-5D-5L). *Qual Life Res* 2011;20(10):1727-36. doi: 10.1007/s11136-011-
- 6 9903-x [published Online First: 2011/04/12]
- 7 87. Snaith RP, Zigmond AS. The hospital anxiety and depression scale. *Br Med J (Clin Res Ed)*
- 8 1986;292(6516):344. doi: 10.1136/bmj.292.6516.344 [published Online First: 1986/02/01]
- 9 88. Ustun TB, Chatterji S, Kostanjsek N, et al. Developing the World Health Organization Disability
- 10 Assessment Schedule 2.0. *Bull World Health Organ* 2010;88(11):815-23. doi:
- 11 10.2471/BLT.09.067231 [published Online First: 2010/11/16]
- 12 89. Lyons KD, Bakitas M, Hegel MT, et al. Reliability and validity of the Functional Assessment of Chronic
- 13 Illness Therapy-Palliative care (FACIT-Pal) scale. *J Pain Symptom Manage* 2009;37(1):23-32.
- 14 doi: 10.1016/j.jpainsymman.2007.12.015 [published Online First: 2008/05/28]
- 15 90. Sewtz C, Muscheites W, Kriesen U, et al. Questionnaires measuring quality of life and satisfaction
- 16 of patients and their relatives in a palliative care setting-German translation of FAMCARE-2
- 17 and the palliative care subscale of FACIT-Pal. *Ann Palliat Med* 2018;7(4):420-26. doi:
- 18 10.21037/apm.2018.03.17 [published Online First: 2018/06/05]
- 19 91. Golicki D, Niewada M, Karlinska A, et al. Comparing responsiveness of the EQ-5D-5L, EQ-5D-3L and
- 20 EQ VAS in stroke patients. *Qual Life Res* 2015;24(6):1555-63. doi: 10.1007/s11136-014-0873-7
- 21 [published Online First: 2014/11/27]
- 22 92. Ludwig K, Graf von der Schulenburg JM, Greiner W. German Value Set for the EQ-5D-5L.
- 23 *Pharmacoeconomics* 2018;36(6):663-74. doi: 10.1007/s40273-018-0615-8 [published Online
- 24 First: 2018/02/21]
- 25 93. Chuang LH, Cohen AT, Agnelli G, et al. Comparison of quality of life measurements: EQ-5D-5L versus
- 26 disease/treatment-specific measures in pulmonary embolism and deep vein thrombosis. *Qual*
- 27 *Life Res* 2019;28(5):1155-77. doi: 10.1007/s11136-018-2081-3 [published Online First:
- 28 2019/01/05]
- 29 94. Pickard AS, Neary MP, Cella D. Estimation of minimally important differences in EQ-5D utility and
- 30 VAS scores in cancer. *Health and quality of life outcomes* 2007;5:70. doi: 10.1186/1477-7525-
- 31 5-70
- 32 95. Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic
- 33 (ROC) curve. *Radiology* 1982;143(1):29-36. doi: 10.1148/radiology.143.1.7063747 [published
- 34 Online First: 1982/04/01]
- 35 96. Baur J, Moreno-Villanueva M, Kotter T, et al. MARK-AGE data management: Cleaning, exploration
- 36 and visualization of data. *Mech Ageing Dev* 2015;151:38-44. doi: 10.1016/j.mad.2015.05.007
- 37 [published Online First: 2015/05/26]
- 38 97. Dereli O, Oguz C, Gonen M. Path2Surv: Pathway/gene set-based survival analysis using multiple
- 39 kernel learning. *Bioinformatics* 2019;35(24):5137-45. doi: 10.1093/bioinformatics/btz446
- 40 [published Online First: 2019/05/31]
- 41 98. Buzdin A, Sorokin M, Garazha A, et al. Molecular pathway activation - New type of biomarkers for
- 42 tumor morphology and personalized selection of target drugs. *Semin Cancer Biol* 2018;53:110-
- 43 24. doi: 10.1016/j.semcancer.2018.06.003 [published Online First: 2018/06/24]
- 44 99. Warsaw G, Greber B, Falk SS, et al. ExprEssence--revealing the essence of differential experimental
- 45 data in the context of an interaction/regulation net-work. *BMC Syst Biol* 2010;4:164. doi:
- 46 10.1186/1752-0509-4-164 [published Online First: 2010/12/02]
- 47 100. Ernst M, Du Y, Warsaw G, et al. FocusHeuristics - expression-data-driven network optimization
- 48 and disease gene prediction. *Sci Rep* 2017;7:42638. doi: 10.1038/srep42638 [published Online
- 49 First: 2017/02/17]
- 50 101. Hanzelmann S, Castelo R, Guinney J. GSEA: gene set variation analysis for microarray and RNA-
- 51 seq data. *BMC Bioinformatics* 2013;14:7. doi: 10.1186/1471-2105-14-7 [published Online First:
- 52 2013/01/18]
- 53 102. Geistlinger L, Csaba G, Santarelli M, et al. Toward a gold standard for benchmarking gene set
- 54 enrichment analysis. *Brief Bioinform* 2020 doi: 10.1093/bib/bbz158 [published Online First:
- 55 2020/02/07]
- 56
- 57
- 58
- 59
- 60



- 1  
2  
3 103. List M, Alcaraz N, Dissing-Hansen M, et al. KeyPathwayMinerWeb: online multi-omics network  
4 enrichment. *Nucleic Acids Res* 2016;44(W1):W98-W104. doi: 10.1093/nar/gkw373 [published  
5 Online First: 2016/05/07]  
6  
7 104. Neto E, Pratap A, Perumal T, et al. Using permutations to assess confounding in machine learning  
8 applications for digital health. *ArXiv* 2018; arXiv:1811.11920 or arXiv:1811.11920v1  
9  
10 105. Sorzano C, Tabas-Madrid D, Nunez F, et al. Sample Size for Pilot Studies and Precision Driven  
11 Experiments. *ArXiv* 2017; arXiv:1707.00222 or arXiv:1707.00222v2  
12  
13 106. Sauerbrei W, Schumacher M. A bootstrap resampling procedure for model building: application  
14 to the Cox regression model. *Stat Med* 1992;11(16):2093-109. doi: 10.1002/sim.4780111607  
15 [published Online First: 1992/12/01]  
16  
17 107. Lin DY. Cox regression analysis of multivariate failure time data: the marginal approach. *Stat Med*  
18 1994;13(21):2233-47. doi: 10.1002/sim.4780132105 [published Online First: 1994/11/15]  
19  
20 108. Binder H, Schumacher M. Allowing for mandatory covariates in boosting estimation of sparse  
21 high-dimensional survival models. *BMC Bioinformatics* 2008;9:14. doi: 10.1186/1471-2105-9-  
22 14 [published Online First: 2008/01/12]  
23  
24 109. Ishwaran H, Kogalur UB, Blackstone EH, et al. Random survival forests. *Ann Appl Stat*  
25 2008;2(3):841-60. doi: 10.1214/08-AOAS169  
26  
27 110. Pi L, Halabi S. Combined Performance of Screening and Variable Selection Methods in Ultra-High  
28 Dimensional Data in Predicting Time-To-Event Outcomes. *Diagn Progn Res* 2018;2 doi:  
29 10.1186/s41512-018-0043-4 [published Online First: 2018/11/06]  
30  
31 111. Ching T, Zhu X, Garmire LX. Cox-nnet: An artificial neural network method for prognosis prediction  
32 of high-throughput omics data. *PLoS Comput Biol* 2018;14(4):e1006076. doi:  
33 10.1371/journal.pcbi.1006076 [published Online First: 2018/04/11]  
34  
35 112. Hao J, Kim Y, Kim TK, et al. PASNet: pathway-associated sparse deep neural network for prognosis  
36 prediction from high-throughput data. *BMC Bioinformatics* 2018;19(1):510. doi:  
37 10.1186/s12859-018-2500-z  
38  
39 113. Yousefi S, Amrollahi F, Amgad M, et al. Predicting clinical outcomes from large scale cancer  
40 genomic profiles with deep survival models. *Sci Rep* 2017;7(1):11707. doi: 10.1038/s41598-  
41 017-11817-6  
42  
43 114. Bass A, Storey J. *bioRxiv* 2019 doi: 10.1101/571992  
44  
45 115. Moeller S, Saul N, Cohen AA, et al. Healthspan pathway maps in *C. elegans* and humans highlight  
46 transcription, proliferation/biosynthesis and lipids. *bioRxiv* 2018  
47  
48 116. Motwani HV, Frostne C, Tornqvist M. Parallelogram based approach for in vivo dose estimation  
49 of genotoxic metabolites in humans with relevance to reduction of animal experiments. *Sci*  
50 *Rep* 2017;7(1):17560. doi: 10.1038/s41598-017-17692-5  
51  
52 117. Kienhuis AS, van de Poll MC, Wortelboer H, et al. Parallelogram approach using rat-human in vitro  
53 and rat in vivo toxicogenomics predicts acetaminophen-induced hepatotoxicity in humans.  
54 *Toxicol Sci* 2009;107(2):544-52. doi: 10.1093/toxsci/kfn237  
55  
56 118. Taroni JN, Grayson PC, Hu Q, et al. MultiPLIER: A Transfer Learning Framework for Transcriptomics  
57 Reveals Systemic Features of Rare Disease. *Cell Syst* 2019;8(5):380-94 e4. doi:  
58 10.1016/j.cels.2019.04.003 [published Online First: 2019/05/24]  
59  
60 119. Schussler-Fiorenza Rose SM, Contrepois K, Moneghetti KJ, et al. A longitudinal big data approach  
for precision health. *Nat Med* 2019;25(5):792-804. doi: 10.1038/s41591-019-0414-6  
[published Online First: 2019/05/10]  
120. Avelar RA, Ortega JG, Tacutu R, et al. A Multidimensional Systems Biology Analysis of Cellular  
Senescence in Ageing and Disease. *bioRxiv* 2019  
121. Demaria M, O'Leary MN, Chang J, et al. Cellular Senescence Promotes Adverse Effects of  
Chemotherapy and Cancer Relapse. *Cancer Discov* 2017;7(2):165-76. doi: 10.1158/2159-  
8290.CD-16-0241 [published Online First: 2016/12/17]  
122. Fulop T, Larbi A, Dupuis G, et al. Immunosenescence and Inflamm-Aging As Two Sides of the Same  
Coin: Friends or Foes? *Front Immunol* 2017;8:1960. doi: 10.3389/fimmu.2017.01960

1  
2  
3  
4  
5 Figure Legends  
6  
7

8  
9 Figure 1: Study design of the SASKit study (human cohort; mouse studies designed to mirror the human  
10 study in part will be presented elsewhere). Predictor and outcome measurements along the time axis  
11 are described.  
12

13  
14 Figure 2: Data analysis plan of the SASKit study (human cohort). Input, methods and output of the  
15 standard (but not the explorative) analyses based on biostatistics and machine learning are described  
16 in detail.  
17

18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For peer review only



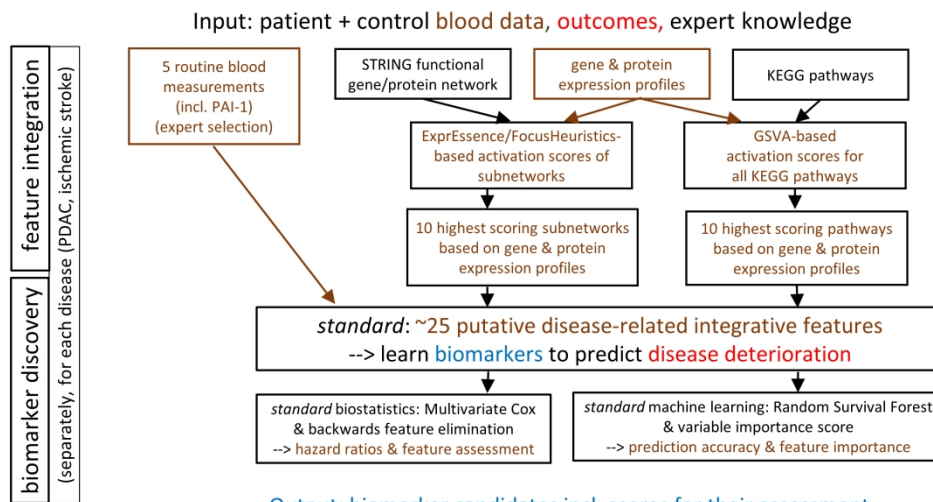
Patient + control, flowchart of activities

|  | month 0                | month 3          | month 6      | month 12   | month 24   | month 36   | month 48   |
|--|------------------------|------------------|--------------|------------|------------|------------|------------|
|  | (for all, by default:) | (patients only:) | (PDAC only:) | (for all:) | (for all:) | (for all:) | (for all:) |
| interview  | ✓                      | ✓                | ✓            | ✓          | ✓          | ✓          | ✓          |
| general data, ECG                                      | ✓                      | ✓                | ✓            | ✓          | ✓          | ✓          | ✓          |
| blood routine  | ✓                      | ✓                | ✓            | ✓          | ✓          | ✓          | ✓          |
| incl. PAI-1  |                        |                  |              |            |            |            |            |
| CA19-9 in patients                                     | (✓)                    | (✓)              |              | (✓)        | (✓)        | (✓)        | (✓)        |
| collection T cells                                     | ✓                      | ✓                |              | ✓          |            |            |            |
| collection serum                                       | ✓                      | ✓                |              | ✓          |            |            |            |
| <b>grip strength</b>                                   | ✓                      | ✓                | ✓            | ✓          | ✓          | ✓          | ✓          |
| <b>clinical performance measurements</b>               | ✓                      | ✓                | ✓            | ✓          | ✓          | ✓          | ✓          |
| <b>patient-reported outcomes (FACIT-PAL: for PDAC)</b> | ✓                      | ✓                | ✓            | ✓          | ✓          | ✓          | ✓          |
|  | (✓)                    | (✓)              | ✓            | (✓)        | (✓)        | (✓)        | (✓)        |

Note: T cells & sera are collected for omics to be thawed & analyzed as follows:  
 in case of PDAC only for month 0; and for month 3 (month 12 is rare),  
 in case of ischemic stroke only for either month 0 or month 3, i.e., for the better NIHSS score; and for month 12.

Study design of the SASKit study (human cohort; mouse studies designed to mirror the human study in part will be presented elsewhere). Predictor and outcome measurements along the time axis are described.

254x142mm (300 x 300 DPI)



Output: biomarker candidates incl. scores for their assessment

explorative: use other features/outcomes/methods; also investigate diseases jointly

Data analysis plan of the SASKit study (human cohort). Input, methods and output of the standard (but not the explorative) analyses based on biostatistics and machine learning are described in detail.

254x142mm (300 x 300 DPI)

# BMJ Open

## Towards biomarkers for outcomes after pancreatic ductal adenocarcinoma and ischemic stroke, with focus on (co-)morbidity and aging / cellular senescence (SASKit): protocol for a prospective cohort study

|                               |  |
|-------------------------------|--|
| Journal:                      | <i>BMJ Open</i>  |
| Manuscript ID                 | bmjopen-2020-039560.R2   |
| Article Type:                 | Protocol   |
| Date Submitted by the Author: | 12-Nov-2020  |
| Complete List of Authors:     | <p>Henze, Larissa; Rostock University Medical Center, Department of Hematology, Oncology and Palliative Medicine, Department of Medicine III, Research Focus Oncology, Rostock University Medical Center</p> <p>Walter, Uwe; Rostock University Medical Center, Department of Child and Adolescence Psychiatry and Neurology, Centre for Transdisciplinary Neurosciences Rostock, Rostock University Medical Center</p> <p>Murua Escobar, Hugo; Rostock University Medical Center, Department of Hematology, Oncology and Palliative Medicine, Department of Medicine III, Research Focus Oncology, Rostock University Medical Center</p> <p>Junghanß, Christian; Rostock University Medical Center, Department of Hematology, Oncology and Palliative Medicine, Department of Medicine III, Research Focus Oncology, Rostock University Medical Center</p> <p>Jaster, Robert; Rostock University Medical Center, Department of Gastroenterology, Research Focus Oncology, Rostock University Medical Center</p> <p>Köhlning, Rüdiger; Rostock University Medical Center, Oscar Langendorff Institute of Physiology, Centre for Transdisciplinary Neurosciences Rostock, Rostock University Medical Center, Ageing of Individuals and Society, Interdisciplinary Faculty, Rostock University</p> <p>Lange, Falko; Rostock University Medical Center, Oscar Langendorff Institute of Physiology, Centre for Transdisciplinary Neurosciences Rostock, Rostock University Medical Center</p> <p>Salehzadeh-Yazdi, Ali; University of Rostock, Department of Systems Biology and Bioinformatics</p> <p>Wolkenhauer, Olaf; University of Rostock, Department of Systems Biology and Bioinformatics, Centre for Transdisciplinary Neurosciences Rostock, Rostock University Medical Center</p> <p>Hamed, Mohamed; Rostock University Medical Center, Institute for Biostatistics and Informatics in Medicine and Ageing Research, Research Focus Oncology, Rostock University Medical Center</p> <p>Barrantes, Israel; Rostock University Medical Center, Institute for Biostatistics and Informatics in Medicine and Ageing Research</p> <p>Palmer, Daniel; Rostock University Medical Center, Institute for Biostatistics and Informatics in Medicine and Ageing Research</p> <p>Möller, Steffen; Rostock University Medical Center, Institute for Biostatistics and Informatics in Medicine and Ageing Research</p> <p>Kowald, Axel; Rostock University Medical Center, Institute for Biostatistics and Informatics in Medicine and Ageing Research</p> |

|                                  |   |
|----------------------------------|---|
|                                  | Heussen, Nicole; RWTH Aachen University, Department of Medical Statistics, Research Focus Oncology, Rostock University Medical Center Fuellen, Georg; Rostock University Medical Center, Institute for Biostatistics and Informatics in Medicine and Ageing Research, Centre for Transdisciplinary Neurosciences Rostock, Rostock University Medical Center, Research Focus Oncology, Rostock University Medical Center, Ageing of Individuals and Society, Interdisciplinary Faculty, Rostock University |
| <b>Primary Subject Heading</b> : | Diagnostics   |
| Secondary Subject Heading:       | Genetics and genomics   |
| Keywords:                        | Health informatics < BIOTECHNOLOGY & BIOINFORMATICS, Immunology < NATURAL SCIENCE DISCIPLINES, Thromboembolism < CARDIOLOGY, Molecular aspects < ONCOLOGY, Stroke < NEUROLOGY   |
|                                  |   |

SCHOLARONE™  
Manuscripts



I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our [licence](#).

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which [Creative Commons](#) licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.



## Towards biomarkers for outcomes after pancreatic ductal adenocarcinoma and ischemic stroke, with focus on (co-)morbidity and aging / cellular senescence (SASKit): protocol for a prospective cohort study

Larissa Henze\*<sup>1,##</sup>, Uwe Walter\*<sup>2,#</sup>, Hugo Murua Escobar<sup>1,##</sup>, Christian Junghanß<sup>1,##</sup>, Robert Jaster<sup>3,##</sup>, Rüdiger Köhling<sup>4,#,###</sup>, Falko Lange<sup>4,#</sup>, Ali Salehzadeh-Yazdi<sup>5</sup>, Olaf Wolkenhauer<sup>5,#</sup>, Mohamed Hamed<sup>6,##</sup>, Israel Barrantes<sup>6</sup>, Daniel Palmer<sup>6</sup>, Steffen Möller<sup>6</sup>, Axel Kowald<sup>6</sup>, Nicole Heussen\*\*<sup>7</sup>, Georg Fuellen\*\*<sup>6,#,##,###</sup>

\*joint first authors

\*\*joint corresponding authors: [nheussen@ukaachen.de](mailto:nheussen@ukaachen.de), [fuellen@uni-rostock.de](mailto:fuellen@uni-rostock.de)

1 Rostock University Medical Center, Department of Medicine, Clinic III, Hematology, Oncology, Palliative Medicine, Rostock, Germany

2 Rostock University Medical Center, Department of Neurology, Rostock, Germany

3 Rostock University Medical Center, Department of Gastroenterology, Rostock, Germany

4 Rostock University Medical Center, Oscar Langendorff Institute of Physiology, Rostock, Germany

5 University of Rostock, Department of Systems Biology and Bioinformatics, Rostock, Germany

6 Rostock University Medical Center, Institute for Biostatistics and Informatics in Medicine and Ageing Research, Rostock, Germany

7 RWTH Aachen, Department of Medical Statistics, Aachen, Germany

# Centre for Transdisciplinary Neurosciences Rostock, Rostock University Medical Center ## Research Focus Oncology, Rostock University Medical Center ### Ageing of Individuals and Society, Interdisciplinary Faculty, Rostock University

### Abstract

**Introduction:** Aging-related processes such as cellular senescence are believed to underlie the accumulation of diseases in time, causing (co-)morbidity, including cancer, thromboembolism and stroke. Interfering with these processes may delay, stop or reverse morbidity. To study the link between (co-)morbidity and aging, by exploring biomarkers and molecular mechanisms of disease-triggered deterioration, we will recruit 50 patients with pancreatic ductal adenocarcinoma, 50 patients with (thromboembolic) ischemic stroke and 50 controls, at Rostock University Medical Center. **Methods and Analysis:** We will gather routine blood data, clinical performance measurements and patient-reported outcomes at up to 7 points in time, alongside in-depth transcriptomics & proteomics at two of the early time points. Aiming for clinically relevant biomarkers, the primary outcome is a composite of probable sarcopenia, clinical performance (described by ECOG Performance Status for patients with pancreatic ductal adenocarcinoma and the Modified Rankin Scale for patients with stroke) and quality of life. Further outcomes cover other aspects of morbidity such as cognitive decline, and of comorbidity such as vascular or cancerous events. The data analysis is comprehensive in that it includes biostatistics & machine learning, both following standard role models & additional explorative approaches. *Prognostic* and *predictive* biomarkers for interventions addressing senescence may become available if the biomarkers that we find are specifically related to aging / cellular senescence. Similarly, *diagnostic* biomarkers will be explored. Our findings will require validation in independent studies, and our dataset shall be useful to validate the findings of other studies. In some of the explorative analyses, we shall include insights from systems biology modelling as well as insights from preclinical animal models. We anticipate that our detailed study protocol and data analysis plan may also guide other biomarker exploration trials. **Ethics and Dissemination:** The study was approved by the local ethics committee (Ethikkommission an der Medizinischen Fakultät der Universität Rostock, A2019-0174), registered at the German Clinical Trials Register (DRKS00021184), and results will be published following standard guidelines.

### Article summary

Strengths and limitations of this study:

- In-depth measurements of both relevant outcomes and potential biomarkers.
- Comparatively low number of participants, for both patients and controls.
- In-depth and detailed data analysis plan.
- Investigation of the deterioration of health and (co-)morbidity, not just of survival.
- Two co-morbid diseases investigated in almost identical ways in two sub-studies.

### Introduction

**Study Rationale and Aims.** The primary aim of the SASKit (“Senescence-Associated Systems diagnostics Kit for cancer and stroke”) study is to discover a set of molecular biomarkers for outcomes after pancreatic ductal adenocarcinoma (PDAC) and ischemic stroke (IS), which are specifically useful to predict disease-triggered deterioration of health (“disease deterioration” for short) in terms of probable sarcopenia<sup>1</sup>, reduced clinical performance and quality of life (QoL). The outcomes also include the (co-)morbidity of vascular events (here defined as stroke, myocardial infarction, and venous or arterial thromboembolism) in patients with PDAC, which are observed frequently apart from sarcopenia. Also included is the (co-)morbidity of any kind of cancer and of cognitive decline. Moreover, we consider mortality, as the most canonical outcome. Following up on the primary aim, we will investigate the nature of the molecular biomarkers to find out whether cellular senescence and other aging-associated processes are contributing to disease deterioration. As a secondary aim, we will search for potential *diagnostic* biomarkers related to cellular senescence and other aging-related processes that may differentiate healthy controls from PDAC or IS patients. Therefore, in the following we motivate our study by describing the prevalence and the outcomes of PDAC and IS, the known predictors of these outcomes, and the specific prevalence of co-morbidity as well as known predictors for this co-morbidity. The role of cellular senescence in aging and disease is described in Box 1. The background of the cancerous and vascular comorbidity is described in Box 2. Importantly, despite differences in disease pathology, dynamics and prognosis, there is a lot of evidence that cellular senescence is an important contributor to disease etiology, progression and consequences for both diseases. Avoiding unclear or circular terminology, we define a biomarker in a very general fashion, simply as a feature (data point)  $f_1$  that successfully predicts another feature  $f_2$  at a later time-point<sup>2</sup>, in a biomedical context. Here, features may be composites, based on the measurement of individual features. Often, feature  $f_1$  refers to molecular data, while feature  $f_2$  refers to phenotypic data, such as clinical outcomes. Ultimately, we aim to identify biomarkers that are easy to measure, and that can then be validated in other studies to predict a clinically relevant outcome. The study design is illustrated in Figure 1, while the data analysis plan is summarized in Figure 2.

**Pancreatic ductal adenocarcinoma: prevalence and outcomes.** The incidence of pancreatic cancer is increasing; in 2017 the global incidence was 5.7 per 100,000 person-years<sup>3</sup>. Age is the most important risk factor, and incidence peaks at 65 to 69 years in males and 75 to 79 years in females<sup>3</sup>. Pancreatic ductal adenocarcinoma (PDAC) is the most common histological type of pancreatic cancer<sup>4</sup>. The disease is characterized by late clinical presentation<sup>5</sup>, early metastases and poor prognosis, with a one-year survival rate in Europe of only 15%<sup>6</sup>. Many patients have unresectable disease at the time of diagnosis, either as locally advanced disease or already with metastases. In these cases, therapy is palliative consisting of chemotherapy and/or best supportive care. Disease deterioration with weight loss and low muscle strength, that is, cachexia and sarcopenia<sup>7</sup>, will follow, for some patients rapidly (within a few weeks) and for others during a longer interval of one or two years. Recent developments in oncology have not shown much benefit in clinical trials of patients with PDAC<sup>8</sup>. Inflammation, desmoplasia and early metastases are deemed responsible for the difficulties in targeting the disease.

1  
2  
3 Moreover, vascular events are frequently observed in the course of PDAC and may contribute to  
4 disease deterioration or early death. Venous thromboembolism is the most common event occurring  
5 in up to 34% of patients with metastatic PDAC<sup>9 10</sup>, but arterial ischemic events, like stroke, are also  
6 reported<sup>11-14 15 16</sup>, see also Box 2. Therefore, deterioration and mortality in PDAC can be explained not  
7 only by tumor progression, but also with other factors like sarcopenia/cachexia and vascular events  
8 contributing as well. Furthermore, we suggest that the underlying cause of all these factors are aging-  
9 related processes such as cellular senescence and chronic inflammation.  
10  
11

12 ***Pancreatic ductal adenocarcinoma: known biomarkers and clinical scores.*** In PDAC patients there is  
13 a lack of established scores describing the risk of disease deterioration and the risk of  
14 sarcopenia/cachexia in particular. Referring to the endpoint of overall survival, some recent studies  
15 tried to establish inflammation-based scores to better characterize outcome in PDAC. In a  
16 retrospective analysis of 386 patients with PDAC of different stages, CRP/Alb ratio, neutrophil-  
17 lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR) and modified Glasgow prognostic score  
18 (mGPS) were studied<sup>17</sup>. In patients with locally advanced and metastatic disease, the CRP/Alb ratio  
19 was an independent factor of poor survival<sup>17</sup>. Another retrospective study evaluating CA19-9, CEA,  
20 CRP, LDH and bilirubin levels in locally advanced and metastatic pancreatic cancer patients treated  
21 with chemotherapy showed an independent prognostic significance for overall survival only for CA 19-  
22 9 decline during treatment<sup>18</sup>. Other studies have evaluated risk factors for thromboembolic events in  
23 pancreatic cancer patients and more generally in patients with cancer<sup>19</sup> (see also Box 2). The “Khorana  
24 score”, developed more than ten years ago, is widely used to estimate venous thromboembolic risk in  
25 the population of cancer patients<sup>20</sup>. This score integrates standard laboratory parameters (platelet  
26 count, hemoglobin, leukocyte count), body mass index (BMI) and the cancer site (with pancreatic  
27 cancer and gastric cancer classified as very high risk). Still, its performance was questioned in a  
28 retrospective cohort of pancreatic cancer patients<sup>21</sup> and in a prospective cohort study of patients with  
29 different cancer types, among them 109 with pancreatic cancer<sup>19</sup>. The clinical association of PDAC,  
30 sarcopenia/cachexia and thromboembolism is well-described<sup>11</sup>, but still not understood in its  
31 pathophysiology<sup>22</sup>. Within the SASKit study we aim to identify biomarkers and molecular mechanisms  
32 contributing to this clinical association, by investigating their relation to clinically relevant outcomes.  
33  
34  
35  
36  
37  
38  
39  
40

41 ***Ischemic stroke, prevalence and outcomes.*** Ischemic stroke (IS) occurs in the German population with  
42 an incidence of 236 per 100,000 per year<sup>23</sup>. The mean age of acute stroke patients is 73-74 years, with  
43 more than 80% of patients being over 60 years old. After a first stroke, nearly 5% of patients suffer a  
44 second stroke within a year. Mortality after IS is about 12% within one year and about 30% within five  
45 years<sup>23</sup>. Mild to moderately disabled stroke survivors showed an elevated prevalence of sarcopenia  
46 >6 months after onset of stroke compared with non-stroke individuals (13.2% vs 5.3%)<sup>24</sup>. The  
47 mechanisms underlying sarcopenia include loss of muscle mass, reduction of fibre cross-sectional area  
48 and increased intramuscular fat deposition occurring between 3 weeks and 6 months after stroke in  
49 both paretic and non-paretic limbs<sup>25</sup>. Comorbid, or subsequent cancer may facilitate sarcopenia after  
50 IS. A US nationwide inpatient sample study reported that 10% of hospitalized IS patients have comorbid  
51 cancer, 16% of them with gastrointestinal cancer and 1% with PDAC, and that this association may be  
52 on the rise<sup>26</sup>. Additionally, within two years after IS, another 2% to 4% of patients receive a new cancer  
53 diagnosis<sup>27-29</sup>. Within the SASKit study we aim to identify biomarkers to predict outcome after IS in  
54 terms of general health state (i.e. sarcopenia, deterioration of clinical performance, cognitive  
55 functioning, frailty) and quality of life, as well as (co-)morbidity, as we do for the PDAC cohort.  
56  
57  
58  
59  
60

1  
2  
3 **Ischemic stroke, known biomarkers and clinical scores.** In an early study of 956 patients with acute IS,  
4 determinants of long-term mortality were age, obesity, cardiac arrhythmias, diabetes mellitus,  
5 coronary heart disease and organic brain syndrome at discharge from hospital; interestingly,  
6 hypercholesterolemia and smoking did not affect long-term outcome<sup>30</sup>. More recent studies uniformly  
7 identified age and stroke severity, usually assessed on the NIHSS or similar scales, as biomarkers of  
8 long-term functional outcome and mortality after stroke<sup>31-32</sup>. Fibrinogen has been related to long-term  
9 outcome after stroke<sup>33-34</sup>. There have been conflicting data on the predictive value of serum bilirubin  
10 levels on the long term risk of cardiovascular disease. While some studies are in favor of a predictive  
11 value<sup>35-37</sup>, others are not<sup>38</sup>. Also, CRP levels have been reported to impact the functional long-term  
12 outcome after IS<sup>39</sup>, and early neurological deterioration after IS has been related to decreasing  
13 albumin levels, elevated CRP and fibrinogen levels<sup>40</sup>. Potential biomarkers for occult cancer in IS  
14 patients include elevated D-dimers, fibrinogen, and CRP; infarction in multiple vascular territories; and  
15 poor nutritional status<sup>41</sup>. Interestingly, IS patients with elevation of at least two of the following  
16 coagulation-related serum markers, that is, D-dimer, prothrombin fragment 1.2, thrombin-  
17 antithrombin complex and fibrin monomer, in the post-acute phase of stroke, were more likely to have  
18 occult cancer or recurrent stroke during follow-up for 1.4±0.8 years<sup>42</sup>. In another study of acute IS  
19 patients, high D-dimer levels at admission were independently associated with recurrent stroke and  
20 all-cause mortality during follow-up for up to 3 years<sup>43</sup>. These findings underpin the idea of shared risk  
21 factors for unfavorable outcomes in IS as well as cancer and they suggest that there may be  
22 coagulation-related biomarkers indicating an early stage of carcinogenesis or stroke (see also Box 2).  
23 Nevertheless, the clinical biomarkers that currently exist for predicting outcome are limited in their  
24 performance and clinical utility, and there is a need to overcome the limitations of current predictive  
25 models<sup>44</sup>.

26  
27  
28  
29  
30  
31  
32 **Box 1: Aging and cellular senescence.** Extra lifetime gained over the last century led to the widespread  
33 emergence of age-related diseases that are rarely seen in younger people. Older patients are thus  
34 more likely to display several comorbidities, making treatment difficult and expensive. Over the last  
35 years, strong evidence has accumulated that the presence of senescent cells (i.e. non-dividing but  
36 secretory, damaged, and metabolically active cells that escape apoptosis) is causally involved in  
37 diseases such as atherosclerosis, cancer, fibrosis, pancreatitis, osteoarthritis, Alzheimer disease and  
38 metabolic disorders<sup>45-46</sup>. Evidence that senescent cells are not only correlated with aging and diseases,  
39 but are also causally involved, comes from recent studies, which transplanted senescent cells from old  
40 into young mice<sup>47</sup>. This resulted in persistent functional impairment as well as spread of cellular  
41 senescence to host tissues. Another strong line of evidence comes from experiments that actually  
42 removed senescent cells from aged mice by senolytics<sup>47-49</sup>. In each case an increase in lifespan and a  
43 delay of typical age related diseases was observed. Most recently, the results of human pilot trials of  
44 putative senolytic treatments in case of idiopathic pulmonary fibrosis and osteoarthritis have been  
45 reported. One team<sup>50</sup> treated idiopathic pulmonary fibrosis patients with dasatinib and quercetin and  
46 demonstrated safety as well as notable improvements in some physical abilities. Furthermore, a  
47 human phase-1 study demonstrated that a senolytic compound, which was applied locally in patients  
48 with osteoarthritis of the knee, was safe and well-tolerated<sup>51</sup>. A clinically meaningful improvement in  
49 several measures, including pain, function, as well as modulation of certain senescence-associated  
50 secretory phenotype (SASP) factors and disease-related biomarkers was observed after a single dose.

51  
52  
53  
54  
55  
56  
57 **Box 2: Cellular senescence and the comorbidity of cancer and vascular events.** Some cancers such as  
58 PDAC can trigger vascular events by hyper-coagulation, reflecting Trousseau's syndrome first reported  
59 150 years ago<sup>11</sup>. In turn, strong associations between coagulation, cellular senescence and the SASP  
60

1  
2  
3 were recently demonstrated <sup>52 53</sup>. While cellular senescence can suppress PDAC and cancerous  
4 proliferation in general, it also triggers tumor progression by fostering inflammatory processes,  
5 including the SASP, while on the other hand, after ischemic stroke, it attenuates recovery <sup>54-58</sup>. For both  
6 diseases, causal influences can be traced back to molecular determinants: PAI-1 (also known as  
7 SERPINE1 and part of the SASP) is involved in cancer-triggered thromboembolism <sup>55 57</sup> and stroke  
8 recovery in animals <sup>59</sup>. Other proteins involved in cellular senescence, specifically inflammatory  
9 cytokines such as IL6, and the lesser known osteopontin and gelsolin, are also markers for both PDAC  
10 and stroke <sup>60-63</sup>. The cyclin-dependent kinase CDK5 <sup>64</sup> is implicated in the progression of PDAC as well  
11 as in the recovery from stroke <sup>58 65</sup>. Moreover, apart from being genetic risk factors <sup>66 67</sup>, the most  
12 prominent drivers of cellular senescence (p16/CDKN2A and p21/CDKN1A) also promote PDAC  
13 progression <sup>68</sup> and endothelial embolic and arteriosclerotic mechanisms of stroke <sup>69</sup>. Finally, two small-  
14 molecule interventions into cellular senescence, fisetin and quercetin, are both potential therapeutic  
15 agents of PDAC and stroke. In case of stroke, the blood-brain-barrier is passed by quercetin which  
16 improves stroke outcome <sup>70</sup>. In case of PDAC it was observed that quercetin inhibits pancreatic cancer  
17 growth *in-vitro* and *in-vivo* <sup>71</sup>. Fisetin is found in various fruits (especially strawberries) and it is  
18 chemically similar to quercetin, with strong putative senolytic effects, extending lifespan of mice even  
19 when intervention with fisetin started only at an advanced age <sup>72</sup>. In a study involving nude mice  
20 implanted with prostate cancer cells, treatment with fisetin significantly retarded tumor growth <sup>73</sup>.  
21 Also, in case of lung cancer, there is evidence for the beneficial effects of fisetin. One study showed  
22 that fisetin provides protection against benzo(a)pyrene [B(a)P]-induced lung carcinogenesis in albino  
23 mice <sup>74</sup> and another *in vivo* study demonstrated the synergistic effects of fisetin and cyclophosphamide  
24 in reducing the growth of lung carcinoma in mice <sup>75</sup>. Several other studies have also demonstrated its  
25 anticarcinogenic, neurotrophic and anti-inflammatory effects that are beneficial in numerous diseases,  
26 including pancreatic cancer and stroke <sup>76</sup>.  
27  
28  
29  
30  
31  
32  
33

---

## 34 Methods

35  
36  
37 The presentation is based on the reporting recommendations for tumor marker prognostic studies  
38 (REMARK), that is, items (1) – (11) of the REMARK checklist <sup>77</sup>. The study design is illustrated in Figure  
39 1, while the data analysis plan is summarized in Figure 2.  
40

### 41 Study design

42  
43 The SASKit (“Senescence-Associated Systems diagnostics Kit for cancer and stroke”) study is designed  
44 as a prospective, observational, cohort study to identify biomarkers for disease deterioration in  
45 patients with PDAC or with IS and, specifically, for the (co-)morbidity of these diseases including  
46 vascular events and sarcopenia following the diagnosis of PDAC as well as cancer and cognitive decline  
47 following IS. All patients will be treated for their diseases in accordance with current guidelines or  
48 therapy standards and at the physician's discretion. Due to the observational study design, regular  
49 treatment of the patient is not affected apart from sampling blood (20 to 80 ml at up to 7 time-points  
50 over the next years). Assessment of disease deterioration will be based on standardized clinical  
51 performance measurements, and patient reported outcomes based on questionnaires (see below for  
52 details). Additionally, data from clinical charts and information from the general practitioner will be  
53 collected. The SASKit study is divided into two subtrials with a common control group, both featuring  
54 essentially the same outcomes, predictor measurements and data analysis approaches.  
55  
56  
57

### 58 Patient and Public Involvement

59  
60 It was not possible to involve patients or the public in the design of the study.



### Characteristics of participants (patients and controls)

In the first subtrial (PDAC-subtrial), patients with an initial diagnosis of PDAC in locally advanced or metastatic stage without previous systemic therapy will be considered for enrolment, whereas patients with a (thromboembolic) IS of the supratentorial brain region within the past 3 to 10 days, with a definitive brain infarction volume >10 ml in an assessment by magnetic resonance imaging (MRI) will be considered for the second subtrial (IS-subtrial). Except for some explorative analyses, the subtrials will be analyzed separately.

Within both subtrials, eligible as controls are those without PDAC or IS and with no other malignant disease or other (hemorrhagic) stroke during the past two years. Potential controls will be recruited from persons who have lived in the same household as the patient within the last 2 years, have a maximum age difference of 12 years and are neither brothers nor sisters (i.e. spouses, second-degree relatives or friends). The controls are selected so that the age and gender structure approximately reflects the age and gender distribution of the patients. Therefore, the age and gender of the patients will be continuously recorded, and the controls selected in such a way that their frequency distribution of gender at any time corresponds approximately to that of the currently recruited patients.

The following criteria lead to exclusion from participation in the study for both patients and controls, *at time of recruitment*:

- previous or current medical tumor therapy
- other cancer within the past 2 years
- previous stroke with persistent deficit
- myocardial infarction within the past 2 years
- therapeutic anticoagulation within the past 2 years for longer than 1 month
- pre-existing dementia
- chronic heart failure stage NYHA IV
- terminal renal insufficiency with hemodialysis
- known HIV infection
- known active hepatitis C
- pregnancy
- age < 18 years.

Both subtrials will be implemented according to the same standardized protocol. After written informed consent of each participant, patients will be followed up at 3, 12, 24, 36 and 48 months after their inclusion in the trial, whenever possible. The PDAC-subtrial includes an additional time-point for examinations at 6 months after inclusion, given that mortality due to PDAC is expected to be accelerated as compared to IS. Controls will be followed up at 12, 24, 36, 48 months.

The study is expected to start in the second quarter of 2020 and will finish with the last participant's follow up at 48 months. Until that time, we expect that 50 PDAC patients, 50 IS patients, and 50 controls participated in the trial. The study will be conducted at the Rostock University Medical Center (UMR), Germany at Clinic III - Hematology, Oncology, Palliative Medicine and at the Department of Neurology; the institutions of the other co-authors are supporting the study in a variety of ways. The

1  
2  
3 study is registered at German Clinical Trials Register (DRKS00021184) and will be conducted following  
4 ICH-GCP.  
5

#### 6 General health- and disease-related and demographic data 7

8 General data of the study participants will be recorded at the beginning of the study (“month 0”) and  
9 consist of the following: age, sex, BMI, temperature, blood pressure, heart rate (ECG). Furthermore,  
10 through interviews the following additional data will be recorded: vascular risk factors (arterial  
11 hypertension, diabetes, hyperlipidaemia, smoking habits), history of vascular events (stroke,  
12 myocardial infarction, venous or arterial thromboembolism), atrial fibrillation, history of cancer,  
13 current medication, surgery or blood transfusions in the past three months and vascular or cancerous  
14 events affecting any first-degree relatives. These data may provide influential factors for explorative  
15 analyses, or be employed to interpret and discuss the results of the study.  
16  
17

#### 18 Blood sampling 19

20 Blood sampling will be done in a standardized fashion, that is, fasting and between 8 and 10 am, for all  
21 assays. Routine blood parameters will be recorded at the time-points described above (months 0 to  
22 48). These consist of differential blood count, reticulocytes, INR (International normalized ratio of  
23 prothrombin time), partial thromboplastin time, D-dimers, fibrinogen, factor XII, albumin, bilirubin,  
24 LDH, high-sensitive CRP, CA19-9, cholesterol, and HbA1c. Among the standard measurements, we also  
25 measure the liver parameters ALT, AST and AP as surrogate markers of liver disease.  
26  
27

28 Experimental blood analysis (PAI-1 and omics) will be done for patients at month 0 in case of PDAC, at  
29 month 0 or at month 3 in case of stroke (where the 3-month time point is taken if it reflects a better  
30 state of the patient as described by the NIHSS) (“baseline”). It will furthermore be repeated at month  
31 3 in the case of PDAC, and at month 12 in the case of stroke (“landmark”). For controls, the  
32 experimental blood analysis will be carried out at month 0 and at month 12, assuming that for these,  
33 data do not change much in the 3 months after baseline. The justification for taking the better clinical  
34 state in case of stroke is the maximization of differences with the month 12 follow-up data. In terms  
35 of practicality (being able to calculate a biomarker signature sooner), however, the state at month 0  
36 should be selected for all stroke patients. Since the blood sample will be taken pre-processed and  
37 frozen at month 0 in all cases, we are in principle able to perform the experimental blood analysis for  
38 all stroke patients at month 0, and we can do this analysis in retrospect if deemed necessary. We also  
39 take blood of PDAC patients at month 12, to have the option to do an experimental blood analysis  
40 based on these samples, if deemed useful. In the following we will refer to the *baseline* time-point  
41 (month 0, or month 3 in cases of stroke patients that improved) and the *landmark* time-point (month  
42 3 for PDAC patients and month 12 for stroke patients and controls). The experimental blood analysis  
43 is done earlier for PDAC because of high expected mortality within the first year.  
44  
45  
46  
47

48 The experimental blood analysis includes PAI-1 (see *Box 2*) as well as high-throughput (omics) analyses,  
49 that is, transcriptomics and proteomics analysis in T cells and proteomics of serum. T cells are of  
50 interest because these cells were reported to carry the strongest signal with respect to cellular  
51 senescence, based on the marker p16<sup>78</sup>. We intend to measure gelsolin and osteopontin as well,  
52 provided that sufficiently standardized assays become available in due time; the blood collected for  
53 this measurement shall otherwise be used to measure cytokines/chemokines such as IL6, IL8 and TNF $\alpha$ ,  
54 which are part of the SASP, by ELISA assays. At time of writing, we do not yet have reliable estimates  
55 on the amount of blood cells still available for measuring protein expression, so an antibody-based  
56 protein array (in case of low amounts), or mass spectrometry (in case of sufficiently high amounts) will  
57 be used alternatively. For the blood serum, we intend to use the same protein measurement method.  
58 In the default case of a protein array, we plan to use the novel but dedicated “Senescence Associated  
59  
60

1  
2  
3 Secretary Phenotype (SASP) Antibody Sampler Kit” (consisting of approx. 10 SASP-related proteins  
4 being measured; Cell Signaling Technology) for both cellular and serum proteomics. Further  
5 exploratory molecular analyses not (yet) funded but permitted based on the ethics approval include  
6 the following: single-cell analyses of blood, methylation assays for calculating epigenetic clocks <sup>79</sup>,  
7 genetics by SNP array or whole-genome sequencing, and telomere length. A separate ethics approval  
8 was granted for an optional skin biopsy; skin microbiome analyses are planned as well. More  
9 specifically, participants have the option to provide a skin biopsy of 5 mm from an area that is not  
10 usually visible. We expect that about 30-50% of the participants will opt in. We keep the biopsy in  
11 culture for several days and divide it into several pieces. Using these, we measure biomarkers of  
12 cellular senescence (specifically, senescence-associated  $\beta$ -galactosidase, which cannot easily be  
13 measured in blood) and we treat some pieces with compounds that may affect cellular senescence,  
14 such as quercetin or fisetin. Moreover, we plan to sample the microbiome of the forehead using a  
15 standard swab. This is a very simple procedure, motivated by the claim that a competitive epigenetic  
16 aging clock can be based on such a sample <sup>80</sup>.

20  
21 Blood sample processing for the experimental analysis will be performed according to standard  
22 operating procedures (SOP) at the research laboratory of Clinic III - Hematology, Oncology, Palliative  
23 Medicine. The procedures include flow cytometric control of the sampling quality including distribution  
24 of cell types and vitality as performed in routine diagnostics. Isolation of peripheral blood mononuclear  
25 cells (PBMCs) will also be performed following the SOP used by the laboratory in routine diagnostics.  
26 T Cell separation will be performed according to an established work flow based on magnetic bead  
27 purification via Miltenyi MACS following manufacturer’s instructions. T-Cell fraction purity as well as  
28 vitality will then be verified by flow cytometric analyses as described above. Nucleic acid isolation as  
29 well as protein isolation will be further performed according to the SOP of the research laboratory  
30 performed using column separation (Qiagen, Hilden Germany). RNA integrity values (RIN) will be  
31 analysed using an Agilent Scientific Instruments Bioanalyzer as instructed by the manufacturer. RIN  
32 values above 6 will qualify for RNAseq or Clariom D Array analyses; for RNAseq average reads per  
33 sample will be set at approx. 40 x 10e6.

### 37 Clinical performance measurements and patient-reported outcomes

39 At baseline and at each follow-up, handgrip strength (“grip strength” for short) is measured using a  
40 digital hand dynamometer (Jamar Plus). The test is performed while sitting comfortably, shoulder  
41 adducted, elbow placed on the tabletop and flexed to 90 degrees, with the forearm and wrist in a  
42 neutral position <sup>81</sup>. The highest value of three measurements of maximal isometric contraction of the  
43 dominant hand, or if paralyzed due to IS, contraction of the unaffected hand, is documented in kg.  
44 Further, the following clinical performance measurements are evaluated by the study physician or  
45 study nurse according to standard protocols: ECOG Performance Status (ECOG PS) <sup>82</sup>, modified Rankin  
46 Scale (mRS) <sup>83</sup>, Canadian Study on Health & Aging Clinical Frailty Scale (CSHA-CFS) <sup>84</sup>, NIH-Stroke Scale  
47 (NIHSS) <sup>85</sup>, Montreal Cognitive Assessment (MOCA) <sup>86</sup>. All raters are certified for the applicable scores  
48 (mRS, NIHSS, MOCA). Patient-reported outcomes (measured by questionnaires) are the following: EQ-  
49 5D-5L and EQ-VAS (generic evaluation of QoL in 5 domains and overall on a visual analog scale) <sup>87</sup>,  
50 HADS-D (evaluation of anxiety and depression) <sup>88</sup>, WHODAS 2.0 (WHO Disability Assessment Schedule)  
51 <sup>89</sup>, PASE (physical activity scale for the elderly) <sup>90</sup>, and, for patients with PDAC, FACIT-Pal (evaluating  
52 QoL with focus on palliative symptoms and needs) <sup>91 92</sup>. All questionnaires are administered following  
53 the suppliers’ instructions.

### 57 Follow up data

59 Apart from the clinical and patient-reported outcomes, further follow-up data are BMI, temperature,  
60 blood pressure, heart rate (ECG), atrial fibrillation, current medication, tumor treatment, comorbidity

(any vascular or cancer event), hospital admissions or palliative care. Additionally, based on clinical charts and information from the general practitioner, we will record medication, (co-)morbidity and mortality. Just like the general health- and disease-related and demographic data recorded at time of recruitment, these data may provide influential factors for explorative analyses, or be employed to interpret and discuss the results of the study.

## Endpoints

In both subtrials, the primary endpoint is a composite measure of “disease deterioration” defined as the *first* occurrence within a follow-up interval of at least one of the following.

- a. Sarcopenia, measured by grip strength less than 27 kg for males and less than 16 kg for females (according to the revised European consensus, EWGSOP2<sup>1</sup>).
- b. Deterioration of clinical performance, that is, of the ECOG PS by at least two points (PDAC-subtrial), or of the mRS by at least one point (IS-subtrial).
- c. Deterioration of QoL, described as a reduction of the EQ-5D-5L by at least 0.07 in the index score, **and** deterioration of at least 7 points in the EQ-VAS (ranging from 0-100).

Deterioration will be considered between baseline (month 0) and the respective landmark (follow-up) investigation. As described above, for patients with IS who have improved their condition (measured by NIHSS) within the first 3 months, this time point (month 3) will be used as a baseline instead. Item (a) is the deterioration from “no sarcopenia” to “probable sarcopenia” as defined by current consensus<sup>1</sup>. Grip strength has been widely used for assessing muscle strength, which is currently used as the most reliable measure of muscle function, loss of which indicating sarcopenia<sup>1</sup>. ECOG PS is established in describing the general condition of patients with cancer, whereas mRS is established in patients with stroke. Death is reflected by both scores as ECOG PS of 5 or mRS of 6, and it will always consider death from any cause. The EQ-5D-5L evaluates QoL in five dimensions (mobility, self-care, usual activity, pain/discomfort, and anxiety/depression), all relevant for patients with PDAC and IS. Furthermore, it is a generic score so that results will be comparable for different diseases (as recently described in patients with stroke<sup>93</sup> and for the general population<sup>94</sup>). Even though disease-specific scores might evaluate symptom burden in even more detail, the EQ-5D-5L was recently shown to be comparable to QoL scores developed specifically for pulmonary embolism and deep vein thrombosis (that is, PEmb-QoL, VEINES-QOL/Sym and PACT-Q2) in terms of acceptability, validity and responsiveness<sup>95</sup>. A clinical deterioration in EQ-5D-5L is described as a minimal important difference in the range from 0.07 to 0.09 index points and in VAS from 7 to 10 points<sup>96</sup>, which is the basis for the definition of item (c). Controls reach their endpoint by the same definition as the subcohort for which they serve as control; in any integrative analysis of both subtrials, a deterioration of the mRS by at least one point will be used as the criterion (instead of ECOG PS), because stroke patients in general have a slower deterioration than PDAC patients, and controls naturally have the slowest expected deterioration.

The primary composite endpoint and all secondary endpoints will be evaluated in a first analysis, based on data obtained until summer 2021, and in a second analysis, based on data obtained until summer 2023, and in a third analysis at the end of the study. The second analysis may be delayed until data of 90% of the study participants are available (at least including the month 12 follow-up) and it may then constitute the “main” analysis of the study. To address potential impacts of COVID-19 on the primary and secondary endpoints, the typical COVID-19 symptoms as well as confirmed diagnosis of COVID-19 are recorded for all study participants at each study visit. In addition, at month 12 the presence of serum anti-SARS-CoV-2 IgG antibodies will be analysed.

The following secondary endpoints will be evaluated:

- each component of the primary endpoint (separately);
- occurrence of disease-specific (co-)morbidity, as follows
  - new vascular events (stroke, myocardial infarction, venous or arterial thromboembolism), specifically in patients with PDAC;
  - new cancer, specifically in patients with IS;
  - probable sarcopenia (based on grip strength);
  - cognitive decline (deterioration of MOCA by 3 points from best value at baseline);
- frailty, defined as a CSHA-CFS level of 6, 7, or 8;
- all-cause mortality.

Further, a sum-score summarizing all measurements of phenotypic variables (grip strength, clinical performance measurements, comorbid events, mortality) will be considered as a surrogate for “aging”, normalizing all continuous-scaled components in order to obtain a common scale with an average of zero and standard deviation of one. The components of the sum-score will all be given equal weight.

### Predictors

While all phenotypic features (grip strength, clinical performance, patient reported outcomes, comorbid events, mortality) are contributing to the definition of endpoints (as dependent variables/parameters), all routine and experimental blood features (PAI-1, omics) are considered to be potential predictors; these are also called the independent variables/parameters. This delineation is justified by (a) the paradigm that (clinical) relevance is tied to high-level phenotypes describing health and survival, specifically including QoL <sup>2</sup>, and (b) the goal of developing a “senescence-associated systems diagnostics kit” that includes a careful selection of biomarkers contributing, as much as possible, also to molecular-mechanistic insights into PDAC, IS and their (co-)morbidity, which we hypothesize to be related to cellular senescence and aging. Age and gender will be included as mandatory covariates (also termed confounders, that is, predictors which we do not aim to explore, or which we wish to improve upon) in all statistical models. Further covariates are smoking, liver dysfunction or disease, the baseline NIHSS score in case of IS, as well as locally-advanced vs metastatic PDAC and modality of treatment in case of PDAC. As described, the successful predictors identified by our study, following the statistical analyses outlined below, are called biomarkers; we wish to stress that these are only *candidates* for the ultimate goal of *clinically validated biomarkers*; in particular, they still need to be validated in further studies (based, e.g., on other cohorts). A set of biomarkers is also called a biomarker signature.

### Blinding and pseudonymization

No blinding will be done during the study. However, the primary composite endpoint will be documented without subjective influence due to standardized definitions. Thus, detection bias will be kept at a minimal extent. Furthermore, information bias will be minimized as we will use simple measurements, which are applied in daily practice or are self-reported and easy to perform (e.g. EQ-5D-5L). The rigorous inclusion of all eligible patients within the recruitment period will help to minimize selection bias. All patient data are pseudonymized to all investigators except for the attending physician and study nurse. Since all major data analyses are based on known information about the outcomes (e.g., supervised machine learning with cross-validation), the data analysis will also be performed based on the pseudonymized data. Protection of personal and clinical data of all patients and controls will follow all relevant legal regulations.

### Sample size



1  
2  
3 No formal sample size calculation was performed a-priori for this observational study. The prevalence  
4 of PDAC combined with the requirement to complete the study within a reasonable timeframe implied  
5 a target of 50 patients per group (PDAC, IS and control group). Nevertheless, a power analysis revealed  
6 that a sample size of 50 patients will have 80% power to detect a significant difference by a non-  
7 parametric Wilcoxon statistic between an AUC of 0.75 for a particular biomarker signature compared  
8 to the null hypothesis value of 0.5 at a significance level of 5% under the assumption that about three  
9 times as many patients will reach the primary endpoint, compared to patients who will not reach the  
10 primary endpoint <sup>97</sup>.  
11  
12

### 13 Data Analysis Plan

14  
15 **General considerations:** The guiding criteria for biomarker identification in the SASKit study are the  
16 maximization of the predictive signal, clinical relevance/utility, biomedical/molecular/clinical  
17 interpretability, and practicality/cost. Given the relatively low number of participants in this in-depth  
18 study, to maximize the signal for the endpoints and predictors given as outlined above, we must aim  
19 to use all available information. Regarding endpoints, whenever possible, we thus wish to consider the  
20 (censored) time-to-event information inherent in the baseline and follow-up examinations, and in the  
21 mortality data. The primary endpoint was defined to integrate expected clinical utility and maximum  
22 signal. In defining the (secondary) endpoints, we considered an array of clinically relevant single  
23 endpoints as well as a sum-score of all phenotypic measurements; we hypothesize that the latter  
24 carries the largest amount of signal. Given the small sample, we cannot set aside an extra validation  
25 dataset. For the predictors considered to be covariates/confounders, please see the section on  
26 “Predictors”, above. The data analysis plan is summarized in Figure 2.  
27  
28  
29  
30  
31

32 **Data quality assessment and cleaning:** The need for (and the amount of) data cleaning cannot easily  
33 be estimated beforehand; we plan to follow the MarkAGE guidelines <sup>98</sup> to deal with missing values,  
34 and to detect and rectify outliers and batch artefacts.  
35

36 **Predictor/Feature integration:** Regarding predictors (features), we first need to remember that we  
37 measure at baseline (at months 0 or 3) and at one landmark (main follow-up, that is, at months 3 or  
38 12). While use of baseline features is unrestricted, use of landmark features is, of course, restricted to  
39 prediction of outcomes after the landmark. Further, we need to handle the high dimensionality of the  
40 omics features. Here, upfront feature integration, e.g., by averaging measurements as described  
41 below, is considered preferable specifically for the high-dimensional omics data, for the following  
42 reasons.  
43  
44  
45

- 46 1) A small feature space allows for an easier understanding and interpretation <sup>99</sup>.
- 47 2) Integrated features can be used as input for both the standard biostatistics and the standard  
48 machine learning parts of the analysis.
- 49 3) Use of few features is more time-tested than newer methods featuring the joint calculation of  
50 the prediction model and the selection of the features, albeit the latter are quite often claimed  
51 to be superior by their developers.
- 52 4) Naturally, feature integration avoids multicollinearity and overfitting, and multiple testing is  
53 less of an issue. This counters the “curse of dimensionality” and “de-noises” the data towards  
54 better prediction performance <sup>99 100</sup>.
- 55 5) Feature integration allows the handling of feature heterogeneity, which in our case refers to  
56 routine blood measurements as well as various omics data types.  
57  
58  
59  
60

- 6) In the *explorative* analyses, systems biology modelling and the parallelogram approach are both supposed to deliver further small sets of integrated, highly informative features, which may, e.g., dominate systems behaviour, or which are believed to translate well from animal models to humans.

While most features will be available for the baseline and the landmark time-point, utilizing baseline data is clinically more useful, simply because the prediction for the endpoint is available much earlier. Nevertheless, in the explorative analyses, we will investigate the predictive power of *changes* in feature measurements from baseline to landmark, given that such changes may be more informative about future disease deterioration (and other endpoints) than just baseline values.

**Specific omics data feature integration:** Notably, we face a heterogeneous “multi-view” dataset, usually referred to as “multi-omics”. Our feature integration approach (see above) is also known as a “late integration” type of analysis, implying that measurements for different omics data types are reduced early on to activation scores for pathways or subnetworks that are then integrated at a “late” level. To calculate the activation scores for subnetworks, we use, by default, the ExprEssence/FocusHeuristics *linkscore*<sup>101 102</sup>, taking the links (gene/protein interactions) from a functional interaction network defaulting to STRING. Our experience with the *linkscore* motivates us to include this method as one of the approaches proposed for feature integration in the following, influencing the calculation of up to 10 features on which the standard biostatistics and machine learning shall be based. Specifically, we take the average expression measurement for all patients (as a list of expression values, one per gene) and the average for all controls (as a list of expression values, one per gene) to calculate a *linkscore* for each STRING interaction, and assemble a “condensed” network including all interactions with a *linkscore* in that percentile for which the 50 highest-scoring interactions are shown. These interactions form subnetworks<sup>103</sup>. We then take the average *linkscore* for each subnetwork as the subnetwork activation score. Alternative methods such as *keypathwayminer* will be used in the exploratory analyses, see below. For the pathways (such as KEGG), we will calculate pathway activation scores using Gene Set Variation Analysis (GSVA)<sup>104</sup>. This method calculates pathway activation scores from expression data, is suited for use with microarray as well as RNAseq data and performed strongly in a recent benchmarking analysis<sup>105</sup>. The GSVA-based pathway activation scores can subsequently be compared between patients and controls in the same way as normal gene expression data, calculating, for each pathway, a fold-change of the pathway activation scores between patients and controls. Here, we average over all patients and over all controls, respectively, using the *limma* R package and adjusting for age and gender of the individual patient/control pathway activation. An example of this approach is given in the GSVA publication, where differential pathway activation was identified between acute lymphoblastic lymphoma and mixed-lineage lymphoma<sup>104</sup>. The major downside of feature integration may be information loss; subsequent statistical and machine-learning-based analyses receive only a tiny fraction of the amount of information that is available in total.

Gene expression data (transcriptomics) will be our preferred omics data type. Nevertheless, proteins are closer to the phenotype than transcripts, so we wish to not ignore these. Therefore, we prepare to deal with both kinds of proteome data that we may expect (see “Experimental blood analyses”, above), as follows.

1. Large-scale data, likely based on mass spectrometry, in the order of hundreds or more proteins that can be identified and measured in all the conditions investigated.

2. Small-scale data, likely based on antibody arrays, in the order of ten proteins or less.

Except for the raw data preprocessing depending on the platform, once log-fold changes describing differential expression are established, we thus expect to handle the large-scale proteome data essentially the same as the transcriptomics data, and the small-scale proteome data similarly to the blood routine data, for cells and serum alike. Overall, the omics data are expected to come along three main coordinates, that is,

1. as blood cell transcriptomics and proteomics as well as serum proteomics;
2. longitudinal in time (for baseline and landmark); and
3. for PDAC, IS and control.

All coordinates can be exploited for differential analyses, even though the PDAC and IS data will be analyzed separately except for some integrative *explorative* analyses (see below). In the *explorative* analyses, the *longitudinal* transcriptomics of the patients and controls will also be analyzed together, see below. For the standard biostatistics and machine learning analyses, we plan to employ 5 approaches to feature integration, each yielding a shortlist of 5 integrated features, as follows.

- 1) **(5 features)** A first shortlist of features will consist of the following expert selection from the routine blood measurements (incl. PAI-1): *neutrophil-lymphocyte-ratio*, *fibrinogen*, *high-sensitive C-reactive protein*, *albumin* and *PAI-1*.
- 2) **(5 features)** For the cellular gene expression measurements, we use ExprEssence/FocusHeuristics (see above) to calculate *the top-5 subnetworks scoring highest*.
- 3) **(5 features)** Again for the cellular gene expression measurements, we use GSVA (see above) to calculate the top-5 most strongly changing pathways as features.
- 4) + 5) **(10 features)**
  - a) In case of dealing with large-scale serum proteomics data, we proceed as in (2) + (3);
  - b) In case of dealing with small-scale serum proteomics data, we proceed as follows:
    - i) if the number of features measured successfully is in the order of 10, we refrain from any processing;
    - ii) if the number of features is in the order of around 10-100, we select the 10 features with the smallest p-values indicating differences between the mean values of patient and control, based on a t-test.

For genomic features as per (2), the feature measurements for an individual patient or control will then be the average linkscores of the 5 selected subnetworks, contrasting each patient with average control data, and each control with average patient data. For genomic features as per (3), the feature measurements for each patient/control will be the GSVA scores of the 5 selected pathways. By construction, we expect the resulting features to reflect the up/downregulation of disease-related transcripts/proteins or pathways/subnetworks. Using the GSVA-based integrated features as input to the biostatistical analyses employing Cox proportional hazard models, we are in fact closely following the "Survival analysis in ovarian carcinoma" example as described in the GSVA publication <sup>104</sup>. Regarding the expert selection from the routine blood measurements, we are aware that some of these features may be considered to have an almost trivial relationship to outcome prediction for the diseases we study; e.g. fibrinogen may correlate strongly with the size of the stroke-damaged brain area and may thus be considered a covariate. However, to our knowledge, none of these features are validated clinical biomarkers, and it is quite possible that a combination of simple biomarkers is key to the best possible prediction. We selected the *neutrophil-lymphocyte-ratio* specifically because it is

1  
2  
3 cheap to measure; it is, however, like many other blood-based features, easily influenced by acute  
4 infection.  
5  
6

7 **Exploratory feature integration:** Apart from the FocusHeuristics/ExprEssence *linkscore*, we employ  
8 alternatives such as *keypathwayminer*<sup>106</sup>. Further, we calculate pathway activation scores for the  
9 following senescence-related KEGG pathways, which include PAI-1 (see the Introduction) but do not  
10 refer to a specific disease, as of February 2020: *Cellular senescence*, *HIF-1 signaling pathway*, *p53*  
11 *signaling pathway*, *Apelin signaling pathway*, *Hippo signaling pathway*, *Complement and coagulation*  
12 *cascades*. “Early integration” by, e.g., first averaging transcript and protein expression on a single-gene  
13 basis, is also planned.  
14  
15  
16

17 **Choice of data analysis methods for biomarker discovery:** We will consider two main approaches of  
18 data analysis, one motivated by statistical methods, the other by machine learning approaches. While  
19 this delineation may ultimately be meaningless, we consider that regression is the core ingredient of  
20 the former, while supervised learning characterizes the latter. We will apply standard methods (mostly  
21 in biostatistics) and explore novel approaches (mostly in machine learning; preserving signal implies a  
22 focus on *supervised* approaches in this case). Data analysis for biomarker *discovery* trials in a *clinical*  
23 setting is usually described with a biostatisticians’ mindset, who also developed methods to cope with  
24 the high dimensionality of omics data (see below). On the other hand, the challenges of omics data  
25 also spurred the recent publication of many methods adopting machine learning, which however did  
26 not yet make it into clinical trial analysis routine, but which we wish to test (see below). We will focus  
27 on methods readily available in SAS or as R packages. Notably, the correct choice of method depends  
28 in part on known unknowns such as the strength of the signal (incl. the amount of missing data) in the  
29 routine blood measurements and the omics.  
30  
31  
32  
33  
34

35 **Prediction model quality measures:** Unlike intervention trials with their highly standardized aim of  
36 establishing a statistically significant superiority (or non-inferiority) of one intervention compared to  
37 another (or to standard of care), observational biomarker trials are a more recent development with  
38 fewer precisely quantified criteria of success, and a stronger need to consider the effect size: even if a  
39 biomarker signature enables a significant improvement in predicting an outcome, raising the accuracy  
40 of the prediction, say, from 70% to 75% may not be clinically meaningful, depending on prevalence of  
41 the condition to be predicted, the cost of the biomarker measurement, etc. We thus aim to identify  
42 biomarkers making a maximum of *difference* in prediction accuracy, if we are able to compare to  
43 established scores (see also below). For the biostatistics part, the concordance statistics (c-index) will  
44 be used as an overall measure of predictive accuracy, and time-dependent ROC curves and AUC will  
45 be used to summarize the predictive accuracy at different cut-off points in time. For the machine  
46 learning part, the cross-validated accuracy and AUC/c-index, following<sup>99</sup>, are used, and to take care of  
47 a potential Simpson’s paradox we will either analyse the data stratified by gender, or we will add such  
48 an analysis and check for consistency. More generally, to investigate the role of confounders (and, if  
49 necessary, to correct for these) in the machine learning part, we wish to use the permutation technique  
50 described<sup>107</sup>. We expect that we can identify a set of biomarkers that affords an accuracy of 75% or  
51 more or an AUC of 0.75 or more in correctly predicting the primary endpoint with a precision of +/-  
52 12%<sup>108</sup>. This estimate of precision is based on half the width of a 95% confidence interval (CI) for a  
53 probability of 75%, by extension of item 6 of the tables of Sorzano et al<sup>108</sup>, which shows precision up  
54 to a sample size of N=30.  
55  
56  
57  
58  
59  
60

1  
2  
3 **Standard biostatistical analyses:** A Cox proportional hazards regression model adjusted for age and  
4 gender will be used to estimate the hazard ratio (HR) and corresponding 95% CI to predict the primary  
5 composite endpoint separately within the PDAC cohort and IS cohort. The 5 shortlists of 5 features  
6 (see above) will be providing the canonical predictors, analyzed together. For selection of the most  
7 important features that might be related to the primary endpoint we will use a procedure proposed  
8 by Sauerbrei et al.<sup>109</sup>, as follows. First, 100 bootstrap samples will be generated. Then, a multivariate  
9 Cox proportional hazards regression model with backward elimination with selection level of 0.05 will  
10 be fitted to each replication of the original data set. In a second step features with a relative selection  
11 frequency of 30% or less over all bootstrap samples will be eliminated. In a third step each feature  $X_i$   
12 for which the hypothesis of independence in combination with a feature  $X_j$  can be rejected will be  
13 eliminated if  $X_i$  is less important when  $X_j$  is included in the model, or if it does not gain importance  
14 when  $X_j$  is excluded from the model. All remaining features will be included in the final model.  
15 Graphical and numerical methods will be performed to establish the validity of the proportionality  
16 assumption<sup>110</sup> in the final model. Results will be reported as p-values, HRs and corresponding 95%-CIs.  
17 A p-value of  $p \leq 0.05$  will be interpreted as indicating statistical significance. From the final model a risk  
18 score will be calculated by multiplying the individual feature measurement of a patient with the  
19 estimated regression coefficient of each feature. The c-index will be used as an overall measure of  
20 predictive accuracy of the resulting score, a time-dependent ROC curve and AUC will be used to  
21 summarize the predictive accuracy of the score at specific times. All secondary endpoints will be  
22 evaluated using the same approach as for the primary endpoint except for the sum-score used as a  
23 surrogate for "aging". For this endpoint, a linear mixed effects model with random intercept and spatial  
24 power covariance structure will be fitted to the data to estimate the progression of "aging". The  
25 covariance structure is chosen to reflect the unequal intervals of follow up investigations. Model  
26 assumptions and model fit will be checked by visual inspection of residuals, and influence diagnostics.  
27 Missing values will be taken into account by a likelihood-based approach within the framework of  
28 mixed linear models with the assumption that missing values occur at random. Results will be reported  
29 as p-value assessed at a level of significance of 5% accompanied by the value of the test statistic and  
30 degrees of freedom. In addition, 95% CIs for the progression (slope) will be provided.

31  
32  
33 **Additional exploratory biostatistical analyses:** Again, the primary composite endpoint as well as all  
34 secondary endpoints will be evaluated separately within the PDAC cohort and IS cohort of the  
35 respective sub-trials. In a first approach, univariate Cox proportional hazard models adjusted for age  
36 and gender will be calculated for each omics feature (R package *survival*) using a cut-off of 0.05 on the  
37 false discovery rate. In a second approach, all omics features will be simultaneously considered in a  
38 multivariate Cox model, adjusted for age and gender. Towards this aim, a component-wise likelihood-  
39 based boosting algorithm proposed by Binder and Schumacher 2008<sup>111</sup> (R package *CoxBoost*) will be  
40 used to develop a biomarker signature.

41  
42  
43 **Standard machine learning:** For the machine learning part, the primary outcome and all secondary  
44 outcomes give rise to an assignment of predictor/feature lists to survival times, one such list per study  
45 participant, for which biomarkers are then learned in a supervised fashion. As described, in the  
46 standard analyses, feature integration (see above) will precede the actual calculation of the model  
47 ("deep" learning approaches that take in "all" features are part of the *exploratory* analyses, see below).  
48 In the same way as the standard biostatistics analyses, the same 5 shortlists of 5 features each (see  
49 above) will be providing the canonical predictors, analyzed together. Exploiting time-to-event  
50



information, we will employ random survival forests (RSF) as described by <sup>112</sup> with the following advantages.

1. RSF can now be considered a time-tested approach, and it was the subject of a recent extensive review <sup>68</sup> and of a systematic comparison with LASSO approaches in the case without feature selection (see item 7 of the tables of Pi *et al* <sup>113</sup> for its competitive performance which is not reflected in their abstract).
2. RSF can also work on essentially all features, without a preceding feature integration/selection step, and then be compared, in the explorative machine learning analyses described below, to survival support vector machines (SSVM) and to a novel method Path2Surv that “conjointly” performs feature selection and model training, see <sup>99</sup>.
3. RSF was recently compared to Cox-nnet <sup>114</sup>, a neural network approach which we consider as very promising for the *exploratory* part, see also below.
4. RSF offers a considerable degree of interpretability, given that RSFs are derived from decision trees.
5. RSF is considered “completely data driven and thus independent of model assumptions” and “in case of high dimensional data, limitations of univariate regression approaches such as overfitting, unreliable estimation of regression coefficients, inflated standard errors or convergence problems do not apply” <sup>68</sup>.

In the machine learning part, we calculate accuracy and AUC/c-index using cross-validation to make the best use of our limited sample size, following the setup of <sup>99</sup> and <sup>113</sup> (who, however, set aside separate validation datasets), and we assess the features as biomarkers by ranking them by their variable importance score.

**Additional exploratory machine learning:** Apart from the more time-tested standard machine learning described above, we will also explore methods that were proposed recently, for which it is less straightforward to tell whether these methods are fit-for-purpose in our case, even though they are usually claimed to be superior by their developers based on some test/validation data sets. Specifically, as mentioned above, we expect to test Path2Surv and SSVM <sup>99</sup> as well as Cox-nnet <sup>114</sup> (without prior feature integration); the latter in particular promises a high degree of interpretability. We further explore CNet (employing the censored-data variant), for interpretable biomarkers. We also plan to employ the PASNet <sup>115</sup>, SurvivalNet <sup>116</sup> and SVRc <sup>73</sup> packages. The longitudinal transcriptomics of the patients and the controls may also be analyzed integratively based on the “optimal discovery procedure” <sup>117</sup>, considering, however, that landmark feature data can only be used to predict events after the landmark. Finally, we will map the differential omics data onto a human “healthspan pathway map” <sup>118</sup>, that is, a set of clusters/pathways based on health-related genetic data that we assembled recently.

**Explorative systems biology modelling, explorative parallelogram approach and transfer learning:** As mentioned, systems biology modelling and parallelogram <sup>119</sup> <sup>120</sup> extrapolation are supposed to deliver small sets of highly informative features, by contributing features that are dominating model behaviour or that are shown to translate from the SASKit animal model data. Given the comparatively small number of study participants (but in-depth measurements), we also wish to explore “transfer learning”, which aims to utilize large amounts of public knowledge in the form of latent variables. Specifically, we plan to use, and wish to develop further, the Multiplier <sup>121</sup> approach motivated by the



1  
2  
3 analysis of rare-disease data. Multiplier utilizes the RNASeq-based recount2 compendium, and apart  
4 from the functional network and pathway data that we use in the feature selection part, this  
5 compendium is expected to be a main source of biological knowledge that enters the calculations for  
6 biomarker discovery.  
7  
8

9  
10 **Miscellaneous exploratory approaches and discovery of diagnostic biomarkers:** We will also use  
11 unsupervised machine learning to generate descriptive multi-omics correlation networks, as they were  
12 most recently employed by <sup>122</sup>, there supplemented by linear mixed effects models using (un-  
13 )restricted maximum likelihood approaches; in this very recent biomarker discovery trial of similar  
14 design as ours, but with many more longitudinal omics measurement time-points than ours, we could  
15 not identify other biomarker discovery methods being used. If genetic data become available, we will  
16 include these in some analyses; specifically, we will investigate the added value of *expression*  
17 *quantitative trait loci* (eQTL) analyses. PDAC and IS data will be analyzed together in some integrative  
18 *exploratory* analyses. In that case, the occurrence of specific endpoints will be evaluated according to  
19 the group membership (PDAC or IS). This means that in addition to the biomarker signature, a group  
20 variable, indicating PDAC or IS patients, will be included in the analysis, to assess the difference in the  
21 progression of the respective endpoints between PDAC and IS patients. We also wish to compare PDAC  
22 and IS patient data to data of healthy controls (adjusted for age and gender) by means of logistic  
23 regression models with the aim of identifying candidate biomarkers for the diagnosis of the respective  
24 disease; we then specifically investigate the association of these diagnostic biomarker candidates with  
25 cellular senescence and other aging-related processes (see also the next paragraph).  
26  
27  
28  
29  
30

31 **Further analyses, and comparison with existing biomarkers and biomarker signatures:** Towards the  
32 end, we will investigate the overlap for the various biomarker identification approaches we employed,  
33 assuming that the most frequently found biomarkers may be the most robust and valid ones.  
34 Moreover, we will compare with existing biomarkers and signatures. Regarding the prediction of  
35 vascular events, we will specifically calculate the Khorana and related scores <sup>19</sup> for comparison, and  
36 report the difference in performance. Further, for all biomarkers we find, we will check their  
37 association with cellular senescence, by manual inspection, literature investigation, comparison to  
38 CellAge <sup>123</sup> and the SASP Atlas <sup>52</sup> or by formal enrichment analyses if the number of biomarkers is  
39 sufficiently large to do this in a meaningful way. Also, in a final step, we plan to identify and filter out  
40 the biomarkers that are volatile in the controls. In addition, a comparison of the biomarker profiles  
41 before and after the co-morbid event is aimed for. Finally, for publicly available data of other trials  
42 with a sufficient overlap with our predictors, we will use these as validation datasets.  
43  
44  
45  
46  
47

## 48 Discussion

### 49 Limitations

50  
51 Arguably, the most serious limitation of the SASKit study is the low number of participants. We  
52 mentioned above that in the 4-year-time-frame of the entire study, at the Rostock University Medical  
53 Center we cannot expect to recruit many more than the 50 PDAC patients to be included in this study;  
54 we could recruit more stroke patients and more controls, but given the call for proposals that allowed  
55 this exploratory (not confirmatory) study to be applied for and funded, we considered that within a  
56 limited budget, in-depth omics characterization, animal models (to be detailed in a follow up  
57 publication) and a comprehensive data analysis plan including systems biology modelling were  
58 important aspects of our study that we did not want to exclude.  
59  
60

1  
2  
3 The two most obvious risks to the main goal of finding good biomarkers for the primary outcome based  
4 on the standard data analysis are the following. First, we found it hard to estimate the distribution of  
5 events as defined by the primary outcome; we cannot exclude that too many events take place already  
6 at the start of the study, or until the first follow-up, specifically in the PDAC subtrial, limiting the  
7 amount of information available to the subsequent time-to-event analyses. Then again, had we  
8 defined the primary outcome more conservatively, there would have been a chance that not enough  
9 events happen before the end of the study. Second, we could not identify role-model publications  
10 reporting results of biomarker explorations that made use of machine learning methods, except for,  
11 to some extent, Schussler-Fiorenza et al <sup>122</sup>, so that we enter unknown territory to some degree. The  
12 two most obvious risks to our goal of investigating the role of cellular senescence in the (co-)morbidity  
13 of PDAC and IS could be an insufficient prevalence of co-morbid events, and the complex role of  
14 treatment in case of PDAC, where additional cellular senescence is most likely triggered by therapeutic  
15 intervention <sup>124</sup>. Then again, all molecular high-throughput analyses are essentially explorative and we  
16 are open to discovering biomarkers of disease that do *not* relate to any of our pre-specified  
17 hypotheses.  
18  
19  
20  
21

## 22 Implications

23 We designed the SASKit study to synergistically deliver upon multiple aims that we consider to be of  
24 relevance for specific disease prognosis and treatment as well as for primary, secondary and tertiary  
25 prevention. Employing clinical performance measurements and patient-reported outcomes, we aim  
26 for clinical relevance and we suggest that prognostic biomarker signatures for general health and QoL  
27 are perhaps more important than (progression-free) survival, although there is much more data about  
28 the latter. Moreover, good disease treatment options are still lacking for PDAC as well as for stroke,  
29 and the more we find cellular senescence implicated in disease deterioration, at least in a subgroup of  
30 patients with a specific biomarker signature, the more confidently we can suggest, and further explore,  
31 seno-therapeutic interventions for these two diseases.  
32  
33  
34

35 Notably, we are in the process of starting a parallel human study testing, in healthy elderly people,  
36 interventions into cellular senescence, based on *food* rich in seno-interventional compounds, and we  
37 expect that many aspects of the study design presented herein will be adopted in that parallel study.  
38 That study will also investigate aging- and senescence-related outcomes, and as such it can be seen as  
39 a test of a cautious yet potentially very effective approach to primary prevention; if the *diagnostic*  
40 biomarkers we find in the SASKit study relate to cellular senescence, this observation would constitute  
41 further evidence for (cautious) seno-interventions, moving towards a kind of universal approach of  
42 disease prevention by tackling fundamental aging-related processes (see Boxes 1 and 2).  
43  
44

45 Secondary prevention, aiming to reduce the impact of a disease that has already occurred, can  
46 ultimately be supported by the SASKit study, if we can demonstrate, and (in follow up studies) confirm,  
47 a distinctive role of cellular senescence (and/or other aging-related processes such as  
48 inflammation/inflammaging <sup>125</sup>) in disease deterioration as defined here. Finally, evidence for tertiary  
49 prevention by seno-therapeutic intervention, aiming to attenuate the impact of an ongoing disease, is  
50 also an option based on how accurate, relevant and specific our biomarkers will be.  
51  
52

53 Last but not least, we expect that the in-depth molecular analyses that we wish to conduct will provide  
54 mechanistic insights into the etiology of the diseases we study here, which we just see as models for  
55 the investigation of the fundamental role of aging in general, and of cellular senescence in particular,  
56 in disease and dysfunction.  
57  
58  
59  
60

## Ethics and dissemination

The study protocol has been approved by the ethics committee of the UMR (*Ethikkommission an der Medizinischen Fakultät der Universität Rostock, A2019-0174*). Results shall be published after completion of the study, following standard guidelines.

### Abbreviations:

|            |   |
|------------|---|
| ALT        | Alanine Aminotransferase  |
| AP         | Alkaline Phosphatase  |
| AST        | Aspartate Aminotransferase  |
| AUC        | Area Under the Curve  |
| BMI        | Body Mass Index   |
| CA19-9     | Carbohydrate Antigen  |
| CEA        | Carcinoembryonic antigen  |
| CI         | Confidence interval   |
| COVID-19   | Coronavirus disease 2019  |
| CRP        | C-reactive protein  |
| CSHA-CFS   | Canadian Study on Health & Aging Clinical Frailty Scale             |
| ECOG       | Eastern Cooperative Oncology Group                                  |
| EQ-5D-5L   | EuroQoL 5-Dimension 5-Level   |
| EQ-VAS     | EuroQol Visual Analogue Scale                                       |
| FACIT-Pal  | Functional Assessment of Chronic Illness Therapy-Palliative         |
| HADS-D     | Hospital Anxiety and Depression Scale - German Version              |
| HR         | Hazard ratio  |
| INR        | International normalized ratio                                      |
| IS         | Ischemic Stroke   |
| LDH        | Lactate dehydrogenase   |
| MOCA       | Montreal Cognitive Assessment                                       |
| mRS        | Modified Rankin Scale   |
| NIHSS      | NIH-Stroke Scale  |
| NYHA       | New York Heart Association  |
| PASE       | Physical activity scale of the elderly                              |
| PDAC       | Pancreatic Ductal Adenocarcinoma                                    |
| PS         | Performance status  |
| QoL        | Quality of Life   |
| ROC        | Receiver-Operator Characteristic                                    |
| RSF        | Random survival forests   |
| SARS-CoV-2 | Severe acute respiratory syndrome coronavirus 2                     |
| SASKit     | Senescence-Associated Systems diagnostics Kit for cancer and stroke |
| SASP       | Senescence Associated Secretory Phenotype                           |
| WHODAS     | WHO Disability Assessment Schedule                                  |

### Contributorship statement

Conception, writing and revision: Larissa Henze, Uwe Walter, Hugo Murua Escobar, Christian Junghanß, Robert Jaster, Rüdiger Köhling, Falko Lange, Ali Salehzadeh-Yazdi, Olaf Wolkenhauer, Mohamed Hamed, Israel Barrantes, Daniel Palmer, Steffen Möller, Axel Kowald, Nicole Heussen, Georg Fuellen.

1  
2  
3 Specific clinical considerations: Larissa Henze, Uwe Walter.

4  
5 Specific experimental considerations: Hugo Murua Escobar.

6  
7 Data analysis plan: Daniel Palmer, Nicole Heussen, Georg Fuellen.

8  
9 Acquisition of funding: Larissa Henze, Uwe Walter, Hugo Murua Escobar, Christian Junghanß, Robert Jaster, Rüdiger Köhling, Ali Salehzadeh-Yazdi, Olaf Wolkenhauer, Georg Fuellen.

10  
11  
12 Project coordination: Axel Kowald, Georg Fuellen.

### 13 14 Conflict of Interest

15  
16 Dr. Walter reports personal fees from Ipsen Pharma, grants and personal fees from Merz Pharma, personal fees from Allergan, personal fees from Bristol-Myers Squibb, personal fees from Daiichi Sankyo, personal fees from Bayer Vital, personal fees from Boehringer Ingelheim, personal fees from Pfizer, personal fees from Thieme, and personal fees from Elsevier Press, all outside the submitted work. The other authors have nothing to disclose.

### 17 18 19 20 21 22 Funding

23  
24 We acknowledge the financial support by the Federal Ministry of Education and Research (BMBF) of Germany for the SASKit study (FKZ 01ZX1903A). The funder had no role in the design of the study.

### 25 26 27 Data sharing statement

28  
29 No data available.

### 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 References

1. Cruz-Jentoft AJ, Bahat G, Bauer J, et al. Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing* 2019;48(1):16-31. doi: 10.1093/ageing/afy169 [published Online First: 2018/10/13]
2. Fuellen G, Jansen L, Cohen AA, et al. Health and Aging: Unifying Concepts, Scores, Biomarkers and Pathways. *Aging and Disease* 2019;10(4):883-900.
3. Collaborators GBDPC. The global, regional, and national burden of pancreatic cancer and its attributable risk factors in 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Gastroenterol Hepatol* 2019;4(12):934-47. doi: 10.1016/S2468-1253(19)30347-4
4. Kleeff J, Korc M, Apte M, et al. Pancreatic cancer. *Nat Rev Dis Primers* 2016;2:16022. doi: 10.1038/nrdp.2016.22 [published Online First: 2016/05/10]
5. Llop E, P EG, Duran A, et al. Glycoprotein biomarkers for the detection of pancreatic ductal adenocarcinoma. *World J Gastroenterol* 2018;24(24):2537-54. doi: 10.3748/wjg.v24.i24.2537 [published Online First: 2018/07/03]
6. Carrato A, Falcone A, Ducreux M, et al. A Systematic Review of the Burden of Pancreatic Cancer in Europe: Real-World Impact on Survival, Quality of Life and Costs. *J Gastrointest Cancer* 2015;46(3):201-11. doi: 10.1007/s12029-015-9724-1 [published Online First: 2015/05/15]
7. Cruz-Jentoft AJ, Sayer AA. Sarcopenia. *Lancet* 2019;393(10191):2636-46. doi: 10.1016/S0140-6736(19)31138-9 [published Online First: 2019/06/07]
8. Taieb J, Pointet AL, Van Laethem JL, et al. What treatment in 2017 for inoperable pancreatic cancers? *Ann Oncol* 2017;28(7):1473-83. doi: 10.1093/annonc/mdx174 [published Online First: 2017/05/02]
9. Menapace LA, Peterson DR, Berry A, et al. Symptomatic and incidental thromboembolism are both associated with mortality in pancreatic cancer. *Thromb Haemost* 2011;106(2):371-8. doi: 10.1160/TH10-12-0789 [published Online First: 2011/06/30]

10. Grilz E, Posch F, Konigsbrugge O, et al. Association of Platelet-to-Lymphocyte Ratio and Neutrophil-to-Lymphocyte Ratio with the Risk of Thromboembolism and Mortality in Patients with Cancer. *Thromb Haemost* 2018;118(11):1875-84. doi: 10.1055/s-0038-1673401 [published Online First: 2018/10/09]
11. Bonnerot M, Humbertjean L, Mione G, et al. Cerebral ischemic events in patients with pancreatic cancer: A retrospective cohort study of 17 patients and a literature review. *Medicine (Baltimore)* 2016;95(26):e4009. doi: 10.1097/MD.0000000000004009 [published Online First: 2016/07/02]
12. Navi BB, Reiner AS, Kamel H, et al. Association between incident cancer and subsequent stroke. *Ann Neurol* 2015;77(2):291-300. doi: 10.1002/ana.24325 [published Online First: 2014/12/05]
13. Varki A. Trousseau's syndrome: multiple definitions and multiple mechanisms. *Blood* 2007;110(6):1723-9. doi: 10.1182/blood-2006-10-053736 [published Online First: 2007/05/15]
14. Grilz E, Marosi C, Konigsbrugge O, et al. Association of complete blood count parameters, d-dimer, and soluble P-selectin with risk of arterial thromboembolism in patients with cancer. *J Thromb Haemost* 2019;17(8):1335-44. doi: 10.1111/jth.14484 [published Online First: 2019/05/18]
15. Poiree S, Monnier-Cholley L, Tubiana JM, et al. Acute abdominal aortic thrombosis in cancer patients. *Abdom Imaging* 2004;29(4):511-3. doi: 10.1007/s00261-003-0144-5 [published Online First: 2004/03/17]
16. Schattner A, Klepfish A, Huszar M, et al. Two patients with arterial thromboembolism among 311 patients with adenocarcinoma of the pancreas. *Am J Med Sci* 2002;324(6):335-8. doi: 10.1097/00000441-200212000-00009 [published Online First: 2002/12/24]
17. Liu Z, Jin K, Guo M, et al. Prognostic Value of the CRP/Alb Ratio, a Novel Inflammation-Based Score in Pancreatic Cancer. *Ann Surg Oncol* 2017;24(2):561-68. doi: 10.1245/s10434-016-5579-3 [published Online First: 2016/09/22]
18. Haas M, Heinemann V, Kullmann F, et al. Prognostic value of CA 19-9, CEA, CRP, LDH and bilirubin levels in locally advanced and metastatic pancreatic cancer: results from a multicenter, pooled analysis of patients receiving palliative chemotherapy. *J Cancer Res Clin Oncol* 2013;139(4):681-9. doi: 10.1007/s00432-012-1371-3
19. van Es N, Di Nisio M, Cesarman G, et al. Comparison of risk prediction scores for venous thromboembolism in cancer patients: a prospective cohort study. *Haematologica* 2017;102(9):1494-501. doi: 10.3324/haematol.2017.169060 [published Online First: 2017/05/28]
20. Khorana AA, Kuderer NM, Culakova E, et al. Development and validation of a predictive model for chemotherapy-associated thrombosis. *Blood* 2008;111(10):4902-7. doi: 10.1182/blood-2007-10-116327 [published Online First: 2008/01/25]
21. Kruger S, Haas M, Burkl C, et al. Incidence, outcome and risk stratification tools for venous thromboembolism in advanced pancreatic cancer - A retrospective cohort study. *Thromb Res* 2017;157:9-15. doi: 10.1016/j.thromres.2017.06.021 [published Online First: 2017/07/05]
22. Faille D, Bourrienne MC, de Raucourt E, et al. Biomarkers for the risk of thrombosis in pancreatic adenocarcinoma are related to cancer process. *Oncotarget* 2018;9(41):26453-65. doi: 10.18632/oncotarget.25458 [published Online First: 2018/06/15]
23. Stahmeyer J, Stubenrauch S, Geyer S, et al. The frequency and timing of recurrent stroke—an analysis of routine health insurance data. *Dtsch Arztebl Int* 2019;116:711-7.
24. Ryan AS, Ivey FM, Serra MC, et al. Sarcopenia and Physical Function in Middle-Aged and Older Stroke Survivors. *Arch Phys Med Rehabil* 2017;98(3):495-99. doi: 10.1016/j.apmr.2016.07.015 [published Online First: 2016/08/18]
25. Scherbakov N, von Haehling S, Anker SD, et al. Stroke induced Sarcopenia: muscle wasting and disability after stroke. *Int J Cardiol* 2013;170(2):89-94. doi: 10.1016/j.ijcard.2013.10.031 [published Online First: 2013/11/16]



- 1
  - 2
  - 3
  - 4
  - 5
  - 6
  - 7
  - 8
  - 9
  - 10
  - 11
  - 12
  - 13
  - 14
  - 15
  - 16
  - 17
  - 18
  - 19
  - 20
  - 21
  - 22
  - 23
  - 24
  - 25
  - 26
  - 27
  - 28
  - 29
  - 30
  - 31
  - 32
  - 33
  - 34
  - 35
  - 36
  - 37
  - 38
  - 39
  - 40
  - 41
  - 42
  - 43
  - 44
  - 45
  - 46
  - 47
  - 48
  - 49
  - 50
  - 51
  - 52
  - 53
  - 54
  - 55
  - 56
  - 57
  - 58
  - 59
  - 60
26. Sanossian N, Djabiras C, Mack WJ, et al. Trends in cancer diagnoses among inpatients hospitalized with stroke. *J Stroke Cerebrovasc Dis* 2013;22(7):1146-50. doi: 10.1016/j.jstrokecerebrovasdis.2012.11.016 [published Online First: 2012/12/19]
27. Uemura J, Kimura K, Sibazaki K, et al. Acute stroke patients have occult malignancy more often than expected. *Eur Neurol* 2010;64(3):140-4. doi: 10.1159/000316764 [published Online First: 2010/07/30]
28. Cocho D, Gendre J, Boltès A, et al. Predictors of occult cancer in acute ischemic stroke patients. *J Stroke Cerebrovasc Dis* 2015;24(6):1324-8. doi: 10.1016/j.jstrokecerebrovasdis.2015.02.006 [published Online First: 2015/04/18]
29. Selvik HA, Thomassen L, Bjerkreim AT, et al. Cancer-Associated Stroke: The Bergen NORSTROKE Study. *Cerebrovasc Dis Extra* 2015;5(3):107-13. doi: 10.1159/000440730 [published Online First: 2015/12/10]
30. Weitbrecht WU, Kirchhoff D. [Long-term prognosis of cerebral infarct in comparison with a normal population]. *Versicherungsmedizin* 1995;47(2):46-9. [published Online First: 1995/04/01]
31. Meyer S, Verheyden G, Brinkmann N, et al. Functional and motor outcome 5 years after stroke is equivalent to outcome at 2 months: follow-up of the collaborative evaluation of rehabilitation in stroke across Europe. *Stroke* 2015;46(6):1613-9. doi: 10.1161/STROKEAHA.115.009421 [published Online First: 2015/05/09]
32. Drozdowska BA, Singh S, Quinn TJ. Thinking About the Future: A Review of Prognostic Scales Used in Acute Stroke. *Front Neurol* 2019;10:274. doi: 10.3389/fneur.2019.00274 [published Online First: 2019/04/06]
33. Pedersen A, Stanne TM, Redfors P, et al. Fibrinogen concentrations predict long-term cognitive outcome in young ischemic stroke patients. *Res Pract Thromb Haemost* 2018;2(2):339-46. doi: 10.1002/rth2.12078 [published Online First: 2018/07/27]
34. Swarowska M, Polczak A, Pera J, et al. Hyperfibrinogenemia predicts long-term risk of death after ischemic stroke. *J Thromb Thrombolysis* 2014;38(4):517-21. doi: 10.1007/s11239-014-1122-1 [published Online First: 2014/08/12]
35. Perlstein TS, Pande RL, Creager MA, et al. Serum total bilirubin level, prevalent stroke, and stroke outcomes: NHANES 1999-2004. *Am J Med* 2008;121(9):781-88 e1. doi: 10.1016/j.amjmed.2008.03.045 [published Online First: 2008/08/30]
36. Choi Y, Lee SJ, Spiller W, et al. Causal Associations Between Serum Bilirubin Levels and Decreased Stroke Risk: A Two-Sample Mendelian Randomization Study. *Arterioscler Thromb Vasc Biol* 2020;40(2):437-45. doi: 10.1161/ATVBAHA.119.313055 [published Online First: 2019/12/06]
37. Zhong P, Wu D, Ye X, et al. Association of circulating total bilirubin level with ischemic stroke: a systemic review and meta-analysis of observational evidence. *Ann Transl Med* 2019;7(14):335. doi: 10.21037/atm.2019.06.71 [published Online First: 2019/09/03]
38. Jorgensen ME, Torp-Pedersen C, Finer N, et al. Association between serum bilirubin and cardiovascular disease in an overweight high risk population from the SCOUT trial. *Nutr Metab Cardiovasc Dis* 2014;24(6):656-62. doi: 10.1016/j.numecd.2013.12.009 [published Online First: 2014/02/19]
39. Wang L, Li Y, Wang C, et al. C-reactive Protein, Infection, and Outcome After Acute Ischemic Stroke: A Registry and Systematic Review. *Curr Neurovasc Res* 2019;16(5):405-15. doi: 10.2174/1567202616666191026122011 [published Online First: 2019/11/19]
40. Martin AJ, Price CI. A Systematic Review and Meta-Analysis of Molecular Biomarkers Associated with Early Neurological Deterioration Following Acute Stroke. *Cerebrovasc Dis* 2018;46(5-6):230-41. doi: 10.1159/000495572 [published Online First: 2018/12/06]
41. Navi BB, Iadecola C. Ischemic stroke in cancer patients: A review of an underappreciated pathology. *Ann Neurol* 2018;83(5):873-83. doi: 10.1002/ana.25227 [published Online First: 2018/04/11]
42. Ellis D, Rangaraju S, Duncan A, et al. Coagulation markers and echocardiography predict atrial fibrillation, malignancy or recurrent stroke after cryptogenic stroke. *Medicine (Baltimore)* 2018;97(51):e13830. doi: 10.1097/MD.00000000000013830 [published Online First: 2018/12/24]

- 1
- 2
- 3
- 4 43. Nezu T, Kitano T, Kubo S, et al. Impact of D-dimer levels for short-term or long-term outcomes in
- 5 cryptogenic stroke patients. *J Neurol* 2018;265(3):628-36. doi: 10.1007/s00415-018-8742-x
- 6 [published Online First: 2018/01/27]
- 7
- 8 44. Chaudhary D, Abedi V, Li J, et al. Clinical Risk Score for Predicting Recurrence Following a Cerebral
- 9 Ischemic Event. *Front Neurol* 2019;10:1106. doi: 10.3389/fneur.2019.01106 [published Online
- 10 First: 2019/11/30]
- 11
- 12 45. Yanai H, Fraifeld VE. The role of cellular senescence in aging through the prism of Koch-like criteria.
- 13 *Ageing Res Rev* 2018;41:18-33. doi: 10.1016/j.arr.2017.10.004 [published Online First:
- 14 2017/11/07]
- 15
- 16 46. Gonzalez-Meljem JM, Apps JR, Fraser HC, et al. Paracrine roles of cellular senescence in promoting
- 17 tumourigenesis. *Br J Cancer* 2018;118(10):1283-88. doi: 10.1038/s41416-018-0066-1
- 18 [published Online First: 2018/04/20]
- 19
- 20 47. Xu M, Pirtskhalava T, Farr JN, et al. Senolytics improve physical function and increase lifespan in
- 21 old age. *Nat Med* 2018;24(8):1246-56. doi: 10.1038/s41591-018-0092-9 [published Online
- 22 First: 2018/07/11]
- 23
- 24 48. Baker DJ, Childs BG, Durik M, et al. Naturally occurring p16(Ink4a)-positive cells shorten healthy
- 25 lifespan. *Nature* 2016;530(7589):184-9. doi: 10.1038/nature16932 [published Online First:
- 26 2016/02/04]
- 27
- 28 49. Baar MP, Brandt RMC, Putavet DA, et al. Targeted Apoptosis of Senescent Cells Restores Tissue
- 29 Homeostasis in Response to Chemotoxicity and Aging. *Cell* 2017;169(1):132-47 e16. doi:
- 30 10.1016/j.cell.2017.02.031 [published Online First: 2017/03/25]
- 31
- 32 50. Justice JN, Nambiar AM, Tchkonja T, et al. Senolytics in idiopathic pulmonary fibrosis: Results from
- 33 a first-in-human, open-label, pilot study. *EBioMedicine* 2019 doi:
- 34 10.1016/j.ebiom.2018.12.052
- 35
- 36 51. UNITY. UNITY Biotechnology Reports Promising Topline Data from Phase 1 First-in-human Study of
- 37 UBX0101 in Patients with Osteoarthritis of the Knee, 2019.
- 38
- 39 52. Tanaka T, Biancotto A, Moaddel R, et al. Plasma proteomic signature of age in healthy humans.
- 40 *Ageing Cell* 2018;17(5):e12799. doi: 10.1111/accel.12799 [published Online First: 2018/07/12]
- 41
- 42 53. Wiley CD, Liu S, Limbad C, et al. SILAC Analysis Reveals Increased Secretion of Hemostasis-Related
- 43 Factors by Senescent Cells. *Cell Rep* 2019;28(13):3329-37 e5. doi:
- 44 10.1016/j.celrep.2019.08.049 [published Online First: 2019/09/26]
- 45
- 46 54. Childs BG, Gluscevic M, Baker DJ, et al. Senescent cells: an emerging target for diseases of ageing.
- 47 *Nat Rev Drug Discov* 2017;16(10):718-35. doi: 10.1038/nrd.2017.116 [published Online First:
- 48 2017/07/22]
- 49
- 50 55. Hisada Y, Mackman N. Cancer-associated pathways and biomarkers of venous thrombosis. *Blood*
- 51 2017;130(13):1499-506. doi: 10.1182/blood-2017-03-743211 [published Online First:
- 52 2017/08/16]
- 53
- 54 56. Moir JA, White SA, Mann J. Arrested development and the great escape--the role of cellular
- 55 senescence in pancreatic cancer. *Int J Biochem Cell Biol* 2014;57:142-8. doi:
- 56 10.1016/j.biocel.2014.10.018 [published Online First: 2014/12/03]
- 57
- 58 57. Valenzuela CA, Quintanilla R, Moore-Carrasco R, et al. The Potential Role of Senescence As a
- 59 Modulator of Platelets and Tumorigenesis. *Front Oncol* 2017;7:188. doi:
- 60 10.3389/fonc.2017.00188 [published Online First: 2017/09/13]
- 58 58. Posada-Duque RA, Barreto GE, Cardona-Gomez GP. Protection after stroke: cellular effectors of
- 59 neurovascular unit integrity. *Front Cell Neurosci* 2014;8:231. doi: 10.3389/fncel.2014.00231
- 60 [published Online First: 2014/09/02]
- 59 59. Chan SL, Bishop N, Li Z, et al. Inhibition of PAI (Plasminogen Activator Inhibitor)-1 Improves Brain
- 60 Collateral Perfusion and Injury After Acute Ischemic Stroke in Aged Hypertensive Rats. *Stroke*
- 2018;49(8):1969-76. doi: 10.1161/STROKEAHA.118.022056 [published Online First: 2018/07/12]

- 1  
2  
3 60. Garcia-Berrocoso T, Penalba A, Boada C, et al. From brain to blood: New biomarkers for ischemic  
4 stroke prognosis. *J Proteomics* 2013;94:138-48. doi: 10.1016/j.jprot.2013.09.005 [published  
5 Online First: 2013/09/26]  
6  
7 61. Mendioroz M, Fernandez-Cadenas I, Rosell A, et al. Osteopontin predicts long-term functional  
8 outcome among ischemic stroke patients. *J Neurol* 2011;258(3):486-93. doi: 10.1007/s00415-  
9 010-5785-z [published Online First: 2010/10/23]  
10  
11 62. Pan S, Chen R, Brand RE, et al. Multiplex targeted proteomic assay for biomarker detection in  
12 plasma: a pancreatic cancer biomarker case study. *J Proteome Res* 2012;11(3):1937-48. doi:  
13 10.1021/pr201117w [published Online First: 2012/02/10]  
14  
15 63. Poruk KE, Firpo MA, Scaife CL, et al. Serum osteopontin and tissue inhibitor of metalloproteinase 1  
16 as diagnostic and prognostic biomarkers for pancreatic adenocarcinoma. *Pancreas*  
17 2013;42(2):193-7. doi: 10.1097/MPA.0b013e31825e354d [published Online First:  
18 2013/02/15]  
19  
20 64. Alexander K, Yang HS, Hinds PW. Cellular senescence requires CDK5 repression of Rac1 activity.  
21 *Mol Cell Biol* 2004;24(7):2808-19. doi: 10.1128/mcb.24.7.2808-2819.2004 [published Online  
22 First: 2004/03/17]  
23  
24 65. Feldmann G, Mishra A, Hong SM, et al. Inhibiting the cyclin-dependent kinase CDK5 blocks  
25 pancreatic cancer formation and progression through the suppression of Ras-Ral signaling.  
26 *Cancer Res* 2010;70(11):4460-9. doi: 10.1158/0008-5472.CAN-09-1107 [published Online First:  
27 2010/05/21]  
28  
29 66. Akinyemi R, Tiwari HK, Arnett DK, et al. APOL1, CDKN2A/CDKN2B, and HDAC9 polymorphisms and  
30 small vessel ischemic stroke. *Acta Neurol Scand* 2018;137(1):133-41. doi: 10.1111/ane.12847  
31 [published Online First: 2017/10/05]  
32  
33 67. Cremin C, Howard S, Le L, et al. CDKN2A founder mutation in pancreatic ductal adenocarcinoma  
34 patients without cutaneous features of Familial Atypical Multiple Mole Melanoma (FAMMM)  
35 syndrome. *Hered Cancer Clin Pract* 2018;16:7. doi: 10.1186/s13053-018-0088-y [published  
36 Online First: 2018/03/16]  
37  
38 68. Wang T, Notta F, Navab R, et al. Senescent Carcinoma-Associated Fibroblasts Upregulate IL8 to  
39 Enhance Prometastatic Phenotypes. *Mol Cancer Res* 2017;15(1):3-14. doi: 10.1158/1541-  
40 7786.MCR-16-0192 [published Online First: 2016/09/30]  
41  
42 69. Chen J, Huang X, Halicka D, et al. Contribution of p16INK4a and p21CIP1 pathways to induction of  
43 premature senescence of human endothelial cells: permissive role of p53. *Am J Physiol Heart*  
44 *Circ Physiol* 2006;290(4):H1575-86. doi: 10.1152/ajpheart.00364.2005 [published Online First:  
45 2005/10/26]  
46  
47 70. Tressera-Rimbau A, Arranz S, Eder M, et al. Dietary Polyphenols in the Prevention of Stroke.  
48 *Oxidative medicine and cellular longevity* 2017;2017:7467962. doi: 10.1155/2017/7467962  
49  
50 71. Angst E, Park JL, Moro A, et al. The flavonoid quercetin inhibits pancreatic cancer growth in vitro  
51 and in vivo. *Pancreas* 2013;42(2):223-9. doi: 10.1097/MPA.0b013e318264ccae  
52  
53 72. Yousefzadeh MJ, Zhu Y, McGowan SJ, et al. Fisetin is a senotherapeutic that extends health and  
54 lifespan. *EBioMedicine* 2018;36:18-28. doi: 10.1016/j.ebiom.2018.09.015  
55  
56 73. Khan FM, Zubek VB. Support Vector Regression for Censored Data (SVRc): A Novel Tool for Survival  
57 Analysis. Eighth IEEE International Conference on Data Mining. Pisa, Italy, 2008.  
58  
59 74. Ravichandran N, Suresh G, Ramesh B, et al. Fisetin, a novel flavonol attenuates benzo(a)pyrene-  
60 induced lung carcinogenesis in Swiss albino mice. *Food and chemical toxicology : an  
international journal published for the British Industrial Biological Research Association*  
2011;49(5):1141-7. doi: 10.1016/j.fct.2011.02.005  
75. Touil YS, Seguin J, Scherman D, et al. Improved antiangiogenic and antitumour activity of the  
combination of the natural flavonoid fisetin and cyclophosphamide in Lewis lung carcinoma-  
bearing mice. *Cancer Chemother Pharmacol* 2011;68(2):445-55. doi: 10.1007/s00280-010-  
1505-8

- 1  
2  
3 76. Khan N, Syed DN, Ahmad N, et al. Fisetin: a dietary antioxidant for health promotion. *Antioxid Redox Signal* 2013;19(2):151-62. doi: 10.1089/ars.2012.4901 [published Online First: 2012/11/06]
- 4  
5  
6 77. Altman DG, McShane LM, Sauerbrei W, et al. Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK): explanation and elaboration. *PLoS Med* 2012;9(5):e1001216. doi: 10.1371/journal.pmed.1001216 [published Online First: 2012/06/08]
- 7  
8  
9 78. Liu Y, Sanoff HK, Cho H, et al. Expression of p16(INK4a) in peripheral blood T-cells is a biomarker of human aging. *Aging Cell* 2009;8(4):439-48. doi: 10.1111/j.1474-9726.2009.00489.x
- 10  
11  
12 79. Ward-Caviness CK, Huffman JE, Everett K, et al. DNA methylation age is associated with an altered hemostatic profile in a multiethnic meta-analysis. *Blood* 2018;132(17):1842-50. doi: 10.1182/blood-2018-02-831347
- 13  
14  
15 80. Huang S, Haiminen N, Carrieri AP, et al. Human Skin, Oral, and Gut Microbiomes Predict Chronological Age. *mSystems* 2020;5(1) doi: 10.1128/mSystems.00630-19 [published Online First: 2020/02/13]
- 16  
17  
18 81. Sousa-Santos AR, Amaral TF. Differences in handgrip strength protocols to identify sarcopenia and frailty - a systematic review. *BMC Geriatr* 2017;17(1):238. doi: 10.1186/s12877-017-0625-y [published Online First: 2017/10/19]
- 19  
20  
21 82. Oken MM, Creech RH, Tormey DC, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am J Clin Oncol* 1982;5(6):649-55. [published Online First: 1982/12/01]
- 22  
23  
24 83. van Swieten JC, Koudstaal PJ, Visser MC, et al. Interobserver agreement for the assessment of handicap in stroke patients. *Stroke* 1988;19(5):604-7. doi: 10.1161/01.str.19.5.604 [published Online First: 1988/05/01]
- 25  
26  
27 84. Rockwood K, Song X, MacKnight C, et al. A global clinical measure of fitness and frailty in elderly people. *CMAJ* 2005;173(5):489-95. doi: 10.1503/cmaj.050051 [published Online First: 2005/09/01]
- 28  
29  
30 85. Lyden P, Brott T, Tilley B, et al. Improved reliability of the NIH Stroke Scale using video training. NINDS TPA Stroke Study Group. *Stroke* 1994;25(11):2220-6. doi: 10.1161/01.str.25.11.2220 [published Online First: 1994/11/01]
- 31  
32  
33 86. Nasreddine ZS, Phillips NA, Bedirian V, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *J Am Geriatr Soc* 2005;53(4):695-9. doi: 10.1111/j.1532-5415.2005.53221.x [published Online First: 2005/04/09]
- 34  
35  
36 87. Herdman M, Gudex C, Lloyd A, et al. Development and preliminary testing of the new five-level version of EQ-5D (EQ-5D-5L). *Qual Life Res* 2011;20(10):1727-36. doi: 10.1007/s11136-011-9903-x [published Online First: 2011/04/12]
- 37  
38  
39 88. Snaith RP, Zigmond AS. The hospital anxiety and depression scale. *Br Med J (Clin Res Ed)* 1986;292(6516):344. doi: 10.1136/bmj.292.6516.344 [published Online First: 1986/02/01]
- 40  
41  
42 89. Ustun TB, Chatterji S, Kostanjsek N, et al. Developing the World Health Organization Disability Assessment Schedule 2.0. *Bull World Health Organ* 2010;88(11):815-23. doi: 10.2471/BLT.09.067231 [published Online First: 2010/11/16]
- 43  
44  
45 90. Washburn RA, Smith KW, Jette AM, et al. The Physical Activity Scale for the Elderly (PASE): development and evaluation. *J Clin Epidemiol* 1993;46(2):153-62. doi: 10.1016/0895-4356(93)90053-4 [published Online First: 1993/02/01]
- 46  
47  
48 91. Lyons KD, Bakitas M, Hegel MT, et al. Reliability and validity of the Functional Assessment of Chronic Illness Therapy-Palliative care (FACIT-Pal) scale. *J Pain Symptom Manage* 2009;37(1):23-32. doi: 10.1016/j.jpainsymman.2007.12.015 [published Online First: 2008/05/28]
- 49  
50  
51 92. Sewtz C, Muscheites W, Kriesen U, et al. Questionnaires measuring quality of life and satisfaction of patients and their relatives in a palliative care setting-German translation of FAMCARE-2 and the palliative care subscale of FACIT-Pal. *Ann Palliat Med* 2018;7(4):420-26. doi: 10.21037/apm.2018.03.17 [published Online First: 2018/06/05]
- 52  
53  
54 93. Golicki D, Niewada M, Karlinska A, et al. Comparing responsiveness of the EQ-5D-5L, EQ-5D-3L and EQ VAS in stroke patients. *Qual Life Res* 2015;24(6):1555-63. doi: 10.1007/s11136-014-0873-7 [published Online First: 2014/11/27]
- 55  
56  
57  
58  
59  
60



- 1
- 2
- 3
- 4 94. Ludwig K, Graf von der Schulenburg JM, Greiner W. German Value Set for the EQ-5D-5L. *Pharmacoeconomics* 2018;36(6):663-74. doi: 10.1007/s40273-018-0615-8 [published Online First: 2018/02/21]
- 5
- 6
- 7 95. Chuang LH, Cohen AT, Agnelli G, et al. Comparison of quality of life measurements: EQ-5D-5L versus
- 8 disease/treatment-specific measures in pulmonary embolism and deep vein thrombosis. *Qual*
- 9 *Life Res* 2019;28(5):1155-77. doi: 10.1007/s11136-018-2081-3 [published Online First:
- 10 2019/01/05]
- 11 96. Pickard AS, Neary MP, Cella D. Estimation of minimally important differences in EQ-5D utility and
- 12 VAS scores in cancer. *Health and quality of life outcomes* 2007;5:70. doi: 10.1186/1477-7525-
- 13 5-70
- 14 97. Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic
- 15 (ROC) curve. *Radiology* 1982;143(1):29-36. doi: 10.1148/radiology.143.1.7063747 [published
- 16 Online First: 1982/04/01]
- 17 98. Baur J, Moreno-Villanueva M, Kotter T, et al. MARK-AGE data management: Cleaning, exploration
- 18 and visualization of data. *Mech Ageing Dev* 2015;151:38-44. doi: 10.1016/j.mad.2015.05.007
- 19 [published Online First: 2015/05/26]
- 20 99. Dereli O, Oguz C, Gonen M. Path2Surv: Pathway/gene set-based survival analysis using multiple
- 21 kernel learning. *Bioinformatics* 2019;35(24):5137-45. doi: 10.1093/bioinformatics/btz446
- 22 [published Online First: 2019/05/31]
- 23 100. Buzdin A, Sorokin M, Garazha A, et al. Molecular pathway activation - New type of biomarkers for
- 24 tumor morphology and personalized selection of target drugs. *Semin Cancer Biol* 2018;53:110-
- 25 24. doi: 10.1016/j.semcancer.2018.06.003 [published Online First: 2018/06/24]
- 26 101. Warsaw G, Greber B, Falk SS, et al. ExprEssence--revealing the essence of differential
- 27 experimental data in the context of an interaction/regulation net-work. *BMC Syst Biol*
- 28 2010;4:164. doi: 10.1186/1752-0509-4-164 [published Online First: 2010/12/02]
- 29 102. Ernst M, Du Y, Warsaw G, et al. FocusHeuristics - expression-data-driven network optimization
- 30 and disease gene prediction. *Sci Rep* 2017;7:42638. doi: 10.1038/srep42638 [published Online
- 31 First: 2017/02/17]
- 32 103. Stahnke T, Gajda-Derylo B, Jünemann A, et al. Suppression of the TGF- $\beta$  pathway by a macrolide
- 33 antibiotic decreases fibrotic responses by ocular fibroblasts in vitro. *Royal Society Open Science*
- 34 2020;7(9):200441.
- 35 104. Hanzelmann S, Castelo R, Guinney J. GSVA: gene set variation analysis for microarray and RNA-
- 36 seq data. *BMC Bioinformatics* 2013;14:7. doi: 10.1186/1471-2105-14-7 [published Online First:
- 37 2013/01/18]
- 38 105. Geistlinger L, Csaba G, Santarelli M, et al. Toward a gold standard for benchmarking gene set
- 39 enrichment analysis. *Brief Bioinform* 2020 doi: 10.1093/bib/bbz158 [published Online First:
- 40 2020/02/07]
- 41 106. List M, Alcaraz N, Dissing-Hansen M, et al. KeyPathwayMinerWeb: online multi-omics network
- 42 enrichment. *Nucleic Acids Res* 2016;44(W1):W98-W104. doi: 10.1093/nar/gkw373 [published
- 43 Online First: 2016/05/07]
- 44 107. Neto E, Pratap A, Perumal T, et al. Using permutations to assess confounding in machine learning
- 45 applications for digital health. *ArXiv* 2018; arXiv:1811.11920 or arXiv:1811.11920v1
- 46 108. Sorzano C, Tabas-Madrid D, Nunez F, et al. Sample Size for Pilot Studies and Precision Driven
- 47 Experiments. *ArXiv* 2017; arXiv:1707.00222 or arXiv:1707.00222v2
- 48 109. Sauerbrei W, Schumacher M. A bootstrap resampling procedure for model building: application
- 49 to the Cox regression model. *Stat Med* 1992;11(16):2093-109. doi: 10.1002/sim.4780111607
- 50 [published Online First: 1992/12/01]
- 51 110. Lin DY. Cox regression analysis of multivariate failure time data: the marginal approach. *Stat Med*
- 52 1994;13(21):2233-47. doi: 10.1002/sim.4780132105 [published Online First: 1994/11/15]
- 53 111. Binder H, Schumacher M. Allowing for mandatory covariates in boosting estimation of sparse
- 54 high-dimensional survival models. *BMC Bioinformatics* 2008;9:14. doi: 10.1186/1471-2105-9-
- 55 14 [published Online First: 2008/01/12]
- 56
- 57
- 58
- 59
- 60



- 1  
2  
3 112. Ishwaran H, Kogalur UB, Blackstone EH, et al. Random survival forests. *Ann Appl Stat* 2008;2(3):841-60. doi: 10.1214/08-AOAS169
- 4  
5 113. Pi L, Halabi S. Combined Performance of Screening and Variable Selection Methods in Ultra-High  
6 Dimensional Data in Predicting Time-To-Event Outcomes. *Diagn Progn Res* 2018;2 doi:  
7 10.1186/s41512-018-0043-4 [published Online First: 2018/11/06]
- 8  
9 114. Ching T, Zhu X, Garmire LX. Cox-nnet: An artificial neural network method for prognosis prediction  
10 of high-throughput omics data. *PLoS Comput Biol* 2018;14(4):e1006076. doi:  
11 10.1371/journal.pcbi.1006076 [published Online First: 2018/04/11]
- 12  
13 115. Hao J, Kim Y, Kim TK, et al. PASNet: pathway-associated sparse deep neural network for prognosis  
14 prediction from high-throughput data. *BMC Bioinformatics* 2018;19(1):510. doi:  
15 10.1186/s12859-018-2500-z
- 16  
17 116. Yousefi S, Amrollahi F, Amgad M, et al. Predicting clinical outcomes from large scale cancer  
18 genomic profiles with deep survival models. *Sci Rep* 2017;7(1):11707. doi: 10.1038/s41598-  
19 017-11817-6
- 20  
21 117. Bass A, Storey J. *bioRxiv* 2019 doi: 10.1101/571992
- 22  
23 118. Moller S, Saul N, Cohen AA, et al. Healthspan pathway maps in *C. elegans* and humans highlight  
24 transcription, proliferation/biosynthesis and lipids. *Aging (Albany NY)* 2020;12(13):12534-81.  
25 doi: 10.18632/aging.103514 [published Online First: 2020/07/08]
- 26  
27 119. Motwani HV, Frostne C, Tornqvist M. Parallelogram based approach for in vivo dose estimation  
28 of genotoxic metabolites in humans with relevance to reduction of animal experiments. *Sci*  
29 *Rep* 2017;7(1):17560. doi: 10.1038/s41598-017-17692-5
- 30  
31 120. Kienhuis AS, van de Poll MC, Wortelboer H, et al. Parallelogram approach using rat-human in vitro  
32 and rat in vivo toxicogenomics predicts acetaminophen-induced hepatotoxicity in humans.  
33 *Toxicol Sci* 2009;107(2):544-52. doi: 10.1093/toxsci/kfn237
- 34  
35 121. Taroni JN, Grayson PC, Hu Q, et al. MultiPLIER: A Transfer Learning Framework for Transcriptomics  
36 Reveals Systemic Features of Rare Disease. *Cell Syst* 2019;8(5):380-94 e4. doi:  
37 10.1016/j.cels.2019.04.003 [published Online First: 2019/05/24]
- 38  
39 122. Schussler-Fiorenza Rose SM, Contrepois K, Moneghetti KJ, et al. A longitudinal big data approach  
40 for precision health. *Nat Med* 2019;25(5):792-804. doi: 10.1038/s41591-019-0414-6  
41 [published Online First: 2019/05/10]
- 42  
43 123. Avelar RA, Ortega JG, Tacutu R, et al. A Multidimensional Systems Biology Analysis of Cellular  
44 Senescence in Ageing and Disease. *bioRxiv* 2019
- 45  
46 124. Demaria M, O'Leary MN, Chang J, et al. Cellular Senescence Promotes Adverse Effects of  
47 Chemotherapy and Cancer Relapse. *Cancer Discov* 2017;7(2):165-76. doi: 10.1158/2159-  
48 8290.CD-16-0241 [published Online First: 2016/12/17]
- 49  
50 125. Fulop T, Larbi A, Dupuis G, et al. Immunosenescence and Inflamm-Aging As Two Sides of the Same  
51 Coin: Friends or Foes? *Front Immunol* 2017;8:1960. doi: 10.3389/fimmu.2017.01960

## Figure Legends

52  
53 Figure 1: Study design of the SASKit study. Predictor and outcome measurements along the time axis  
54 are described.

55  
56  
57 Figure 2: Data analysis plan of the SASKit study. Input, methods and output of the standard (but not  
58 the explorative) analyses based on biostatistics and machine learning are described in detail.

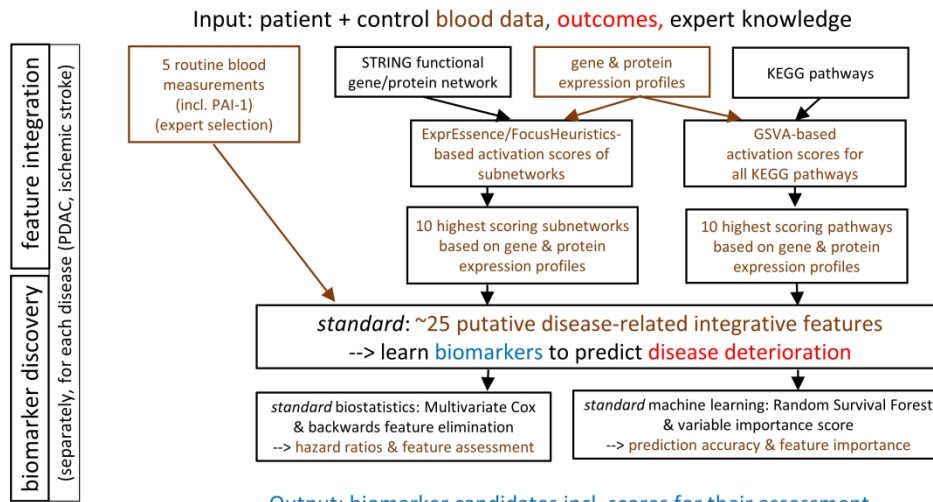
Patient + control, flowchart of activities

|  | month 0                | month 3          | month 6      | month 12   | month 24   | month 36   | month 48   |
|--|------------------------|------------------|--------------|------------|------------|------------|------------|
|  | (for all, by default:) | (patients only:) | (PDAC only:) | (for all:) | (for all:) | (for all:) | (for all:) |
| interview  | ✓                      | ✓                | ✓            | ✓          | ✓          | ✓          | ✓          |
| general data, ECG                                      | ✓                      | ✓                | ✓            | ✓          | ✓          | ✓          | ✓          |
| blood routine  | ✓                      | ✓                | ✓            | ✓          | ✓          | ✓          | ✓          |
| incl. PAI-1  |                        |                  |              |            |            |            |            |
| CA19-9 in patients                                     | (✓)                    | (✓)              |              | (✓)        | (✓)        | (✓)        | (✓)        |
| collection T cells                                     | ✓                      | ✓                |              | ✓          |            |            |            |
| collection serum                                       | ✓                      | ✓                |              | ✓          |            |            |            |
| <b>grip strength</b>                                   | ✓                      | ✓                | ✓            | ✓          | ✓          | ✓          | ✓          |
| <b>clinical performance measurements</b>               | ✓                      | ✓                | ✓            | ✓          | ✓          | ✓          | ✓          |
| <b>patient-reported outcomes (FACIT-PAL: for PDAC)</b> | ✓                      | ✓                | ✓            | ✓          | ✓          | ✓          | ✓          |
|  | (✓)                    | (✓)              | ✓            | (✓)        | (✓)        | (✓)        | (✓)        |

Note: T cells & sera are collected for omics to be thawed & analyzed as follows:  
 in case of PDAC only for month 0; and for month 3 (month 12 is rare),  
 in case of ischemic stroke only for either month 0 or month 3, i.e., for the better NIHSS score; and for month 12.

Study design of the SASKit study (human cohort; mouse studies designed to mirror the human study in part will be presented elsewhere). Predictor and outcome measurements along the time axis are described.

254x142mm (300 x 300 DPI)



Output: biomarker candidates incl. scores for their assessment

explorative: use other features/outcomes/methods; also investigate diseases jointly

Data analysis plan of the SASKit study (human cohort). Input, methods and output of the standard (but not the explorative) analyses based on biostatistics and machine learning are described in detail.

254x142mm (300 x 300 DPI)

# BMJ Open

## Towards biomarkers for outcomes after pancreatic ductal adenocarcinoma and ischemic stroke, with focus on (co-)morbidity and aging / cellular senescence (SASKit): protocol for a prospective cohort study

|                               |   |
|-------------------------------|---|
| Journal:                      | <i>BMJ Open</i>   |
| Manuscript ID                 | bmjopen-2020-039560.R3  |
| Article Type:                 | Protocol  |
| Date Submitted by the Author: | 17-Nov-2020   |
| Complete List of Authors:     | <p>Henze, Larissa; Rostock University Medical Center, Department of Hematology, Oncology and Palliative Medicine, Department of Medicine III, Research Focus Oncology, Rostock University Medical Center</p> <p>Walter, Uwe; Rostock University Medical Center, Department of Child and Adolescence Psychiatry and Neurology, Centre for Transdisciplinary Neurosciences Rostock, Rostock University Medical Center</p> <p>Murua Escobar, Hugo; Rostock University Medical Center, Department of Hematology, Oncology and Palliative Medicine, Department of Medicine III, Research Focus Oncology, Rostock University Medical Center</p> <p>Junghanß, Christian; Rostock University Medical Center, Department of Hematology, Oncology and Palliative Medicine, Department of Medicine III, Research Focus Oncology, Rostock University Medical Center</p> <p>Jaster, Robert; Rostock University Medical Center, Department of Gastroenterology, Research Focus Oncology, Rostock University Medical Center</p> <p>Köhling, Rüdiger; Rostock University Medical Center, Oscar Langendorff Institute of Physiology, Centre for Transdisciplinary Neurosciences Rostock, Rostock University Medical Center, Ageing of Individuals and Society, Interdisciplinary Faculty, Rostock University</p> <p>Lange, Falko; Rostock University Medical Center, Oscar Langendorff Institute of Physiology, Centre for Transdisciplinary Neurosciences Rostock, Rostock University Medical Center</p> <p>Salehzadeh-Yazdi, Ali; University of Rostock, Department of Systems Biology and Bioinformatics</p> <p>Wolkenhauer, Olaf; University of Rostock, Department of Systems Biology and Bioinformatics, Centre for Transdisciplinary Neurosciences Rostock, Rostock University Medical Center</p> <p>Hamed, Mohamed; Rostock University Medical Center, Institute for Biostatistics and Informatics in Medicine and Ageing Research, Research Focus Oncology, Rostock University Medical Center</p> <p>Barrantes, Israel; Rostock University Medical Center, Institute for Biostatistics and Informatics in Medicine and Ageing Research</p> <p>Palmer, Daniel; Rostock University Medical Center, Institute for Biostatistics and Informatics in Medicine and Ageing Research</p> <p>Möller, Steffen; Rostock University Medical Center, Institute for Biostatistics and Informatics in Medicine and Ageing Research</p> <p>Kowald, Axel; Rostock University Medical Center, Institute for Biostatistics and Informatics in Medicine and Ageing Research</p> |

|                                  |  |
|----------------------------------|--|
|                                  | Heussen, Nicole; RWTH Aachen University, Department of Medical Statistics, Research Focus Oncology, Rostock University Medical Center<br>Fuellen, Georg; Rostock University Medical Center, Institute for Biostatistics and Informatics in Medicine and Ageing Research, Centre for Transdisciplinary Neurosciences Rostock, Rostock University Medical Center, Research Focus Oncology, Rostock University Medical Center, Ageing of Individuals and Society, Interdisciplinary Faculty, Rostock University |
| <b>Primary Subject Heading</b> : | Diagnostics  |
| Secondary Subject Heading:       | Genetics and genomics  |
| Keywords:                        | Health informatics < BIOTECHNOLOGY & BIOINFORMATICS, Immunology < NATURAL SCIENCE DISCIPLINES, Thromboembolism < CARDIOLOGY, Molecular aspects < ONCOLOGY, Stroke < NEUROLOGY  |
|                                  |  |

SCHOLARONE™  
Manuscripts





I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our [licence](#).

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which [Creative Commons](#) licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

## Towards biomarkers for outcomes after pancreatic ductal adenocarcinoma and ischemic stroke, with focus on (co-)morbidity and aging / cellular senescence (SASKit): protocol for a prospective cohort study

Larissa Henze\*<sup>1,##</sup>, Uwe Walter\*<sup>2,#</sup>, Hugo Murua Escobar<sup>1,##</sup>, Christian Junghanß<sup>1,##</sup>, Robert Jaster<sup>3,##</sup>, Rüdiger Köhling<sup>4,#,###</sup>, Falko Lange<sup>4,#</sup>, Ali Salehzadeh-Yazdi<sup>5</sup>, Olaf Wolkenhauer<sup>5,#</sup>, Mohamed Hamed<sup>6,##</sup>, Israel Barrantes<sup>6</sup>, Daniel Palmer<sup>6</sup>, Steffen Möller<sup>6</sup>, Axel Kowald<sup>6</sup>, Nicole Heussen\*\*<sup>7</sup>, Georg Fuellen\*\*<sup>6,#,##,###</sup>

\*joint first authors

\*\*joint corresponding authors: [nheussen@ukaachen.de](mailto:nheussen@ukaachen.de), [fuellen@uni-rostock.de](mailto:fuellen@uni-rostock.de)

1 Rostock University Medical Center, Department of Medicine, Clinic III, Hematology, Oncology, Palliative Medicine, Rostock, Germany

2 Rostock University Medical Center, Department of Neurology, Rostock, Germany

3 Rostock University Medical Center, Department of Gastroenterology, Rostock, Germany

4 Rostock University Medical Center, Oscar Langendorff Institute of Physiology, Rostock, Germany

5 University of Rostock, Department of Systems Biology and Bioinformatics, Rostock, Germany

6 Rostock University Medical Center, Institute for Biostatistics and Informatics in Medicine and Ageing Research, Rostock, Germany

7 RWTH Aachen, Department of Medical Statistics, Aachen, Germany

# Centre for Transdisciplinary Neurosciences Rostock, Rostock University Medical Center ## Research Focus Oncology, Rostock University Medical Center ### Ageing of Individuals and Society, Interdisciplinary Faculty, Rostock University

### Abstract

**Introduction:** Aging-related processes such as cellular senescence are believed to underlie the accumulation of diseases in time, causing (co-)morbidity, including cancer, thromboembolism and stroke. Interfering with these processes may delay, stop or reverse morbidity. The aim of this study is to investigate the link between (co-)morbidity and aging, by exploring biomarkers and molecular mechanisms of disease-triggered deterioration in patients with pancreatic ductal adenocarcinoma, and (thromboembolic) ischemic stroke. **Methods and Analysis:** We will recruit 50 patients with pancreatic ductal adenocarcinoma, 50 patients with (thromboembolic) ischemic stroke and 50 controls, at Rostock University Medical Center, Germany. We will gather routine blood data, clinical performance measurements and patient-reported outcomes at up to 7 points in time, alongside in-depth transcriptomics & proteomics at two of the early time points. Aiming for clinically relevant biomarkers, the primary outcome is a composite of probable sarcopenia, clinical performance (described by ECOG Performance Status for patients with pancreatic ductal adenocarcinoma and the Modified Rankin Scale for patients with stroke) and quality of life. Further outcomes cover other aspects of morbidity such as cognitive decline, and of comorbidity such as vascular or cancerous events. The data analysis is comprehensive in that it includes biostatistics & machine learning, both following standard role models & additional explorative approaches. *Prognostic* and *predictive* biomarkers for interventions addressing senescence may become available if the biomarkers that we find are specifically related to aging / cellular senescence. Similarly, *diagnostic* biomarkers will be explored. Our findings will require validation in independent studies, and our dataset shall be useful to validate the findings of other studies. In some of the explorative analyses, we shall include insights from systems biology modelling as well as insights from preclinical animal models. We anticipate that our detailed study protocol and data analysis plan may also guide other biomarker exploration trials. **Ethics and Dissemination:** The study was approved by the local ethics committee (Ethikkommission an der Medizinischen Fakultät der Universität Rostock, A2019-0174), registered at the German Clinical Trials Register (DRKS00021184), and results will be published following standard guidelines.

### Article summary

Strengths and limitations of this study:

- In-depth measurements of both relevant outcomes and potential biomarkers.
- Comparatively low number of participants, for both patients and controls.
- In-depth and detailed data analysis plan.
- Investigation of the deterioration of health and (co-)morbidity, not just of survival.
- Two co-morbid diseases investigated in almost identical ways in two sub-studies.

### Introduction

Pancreatic ductal adenocarcinoma (PDAC) and ischemic stroke (IS) are two aging-associated diseases for which cellular senescence is suspected to play a role regarding their (co-)morbidity. In the following, we outline an observational study of these two diseases, describing the prevalence and outcomes of PDAC and IS, the known predictors of these outcomes, and the specific prevalence of co-morbidity as well as known predictors for this co-morbidity. Moreover, we discuss the role of cellular senescence in aging and disease (specifically, see Box 1), and the background of the cancerous and vascular comorbidity (specifically, see Box 2). We will see that, despite differences in disease pathology, dynamics and prognosis, there is a lot of evidence that cellular senescence is an important contributor to disease etiology, progression and consequences for both diseases.

**Pancreatic ductal adenocarcinoma: prevalence and outcomes.** The incidence of pancreatic cancer is increasing; in 2017 the global incidence was 5.7 per 100,000 person-years<sup>1</sup>. Age is the most important risk factor, and incidence peaks at 65 to 69 years in males and 75 to 79 years in females<sup>1</sup>. Pancreatic ductal adenocarcinoma (PDAC) is the most common histological type of pancreatic cancer<sup>2</sup>. The disease is characterized by late clinical presentation<sup>3</sup>, early metastases and poor prognosis, with a one-year survival rate in Europe of only 15%<sup>4</sup>. Many patients have unresectable disease at the time of diagnosis, either as locally advanced disease or already with metastases. In these cases, therapy is palliative consisting of chemotherapy and/or best supportive care. Disease deterioration with weight loss and low muscle strength, that is, cachexia and sarcopenia<sup>5</sup>, will follow, for some patients rapidly (within a few weeks) and for others during a longer interval of one or two years. Recent developments in oncology have not shown much benefit in clinical trials of patients with PDAC<sup>6</sup>. Inflammation, desmoplasia and early metastases are deemed responsible for the difficulties in targeting the disease. Moreover, vascular events are frequently observed in the course of PDAC and may contribute to disease deterioration or early death. Venous thromboembolism is the most common event occurring in up to 34% of patients with metastatic PDAC<sup>7,8</sup>, but arterial ischemic events, like stroke, are also reported<sup>9-12, 13, 14</sup>, see also Box 2. Therefore, deterioration and mortality in PDAC can be explained not only by tumor progression, but also with other factors like sarcopenia/cachexia and vascular events contributing as well. Furthermore, we suggest that the underlying cause of all these factors are aging-related processes such as cellular senescence and chronic inflammation.

**Pancreatic ductal adenocarcinoma: known biomarkers and clinical scores.** In PDAC patients there is a lack of established scores describing the risk of disease deterioration and the risk of sarcopenia/cachexia in particular. Referring to the endpoint of overall survival, some recent studies tried to establish inflammation-based scores to better characterize outcome in PDAC. In a retrospective analysis of 386 patients with PDAC of different stages, CRP/Alb ratio, neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR) and modified Glasgow prognostic score (mGPS) were studied<sup>15</sup>. In patients with locally advanced and metastatic disease, the CRP/Alb ratio was an independent factor of poor survival<sup>15</sup>. Another retrospective study evaluating CA19-9, CEA,

1  
2  
3 CRP, LDH and bilirubin levels in locally advanced and metastatic pancreatic cancer patients treated  
4 with chemotherapy showed an independent prognostic significance for overall survival only for CA 19-  
5 9 decline during treatment<sup>16</sup>. Other studies have evaluated risk factors for thromboembolic events in  
6 pancreatic cancer patients and more generally in patients with cancer<sup>17</sup> (see also Box 2). The “Khorana  
7 score”, developed more than ten years ago, is widely used to estimate venous thromboembolic risk in  
8 the population of cancer patients<sup>18</sup>. This score integrates standard laboratory parameters (platelet  
9 count, hemoglobin, leukocyte count), body mass index (BMI) and the cancer site (with pancreatic  
10 cancer and gastric cancer classified as very high risk). Still, its performance was questioned in a  
11 retrospective cohort of pancreatic cancer patients<sup>19</sup> and in a prospective cohort study of patients with  
12 different cancer types, among them 109 with pancreatic cancer<sup>17</sup>. The clinical association of PDAC,  
13 sarcopenia/cachexia and thromboembolism is well-described<sup>9</sup>, but still not understood in its  
14 pathophysiology<sup>20</sup>. Within the SASKit study we aim to identify biomarkers and molecular mechanisms  
15 contributing to this clinical association, by investigating their relation to clinically relevant outcomes.  
16  
17  
18  
19

20  
21 **Ischemic stroke, prevalence and outcomes.** Ischemic stroke (IS) occurs in the German population with  
22 an incidence of 236 per 100,000 per year<sup>21</sup>. The mean age of acute stroke patients is 73-74 years, with  
23 more than 80% of patients being over 60 years old. After a first stroke, nearly 5% of patients suffer a  
24 second stroke within a year. Mortality after IS is about 12% within one year and about 30% within five  
25 years<sup>21</sup>. Mild to moderately disabled stroke survivors showed an elevated prevalence of sarcopenia  
26 >6 months after onset of stroke compared with non-stroke individuals (13.2% vs 5.3%)<sup>22</sup>. The  
27 mechanisms underlying sarcopenia include loss of muscle mass, reduction of fibre cross-sectional area  
28 and increased intramuscular fat deposition occurring between 3 weeks and 6 months after stroke in  
29 both paretic and non-paretic limbs<sup>23</sup>. Comorbid, or subsequent cancer may facilitate sarcopenia after  
30 IS. A US nationwide inpatient sample study reported that 10% of hospitalized IS patients have comorbid  
31 cancer, 16% of them with gastrointestinal cancer and 1% with PDAC, and that this association may be  
32 on the rise<sup>24</sup>. Additionally, within two years after IS, another 2% to 4% of patients receive a new cancer  
33 diagnosis<sup>25-27</sup>. Within the SASKit study we aim to identify biomarkers to predict outcome after IS in  
34 terms of general health state (i.e. sarcopenia, deterioration of clinical performance, cognitive  
35 functioning, frailty) and quality of life, as well as (co-)morbidity, as we do for the PDAC cohort.  
36  
37  
38  
39  
40

41  
42 **Ischemic stroke, known biomarkers and clinical scores.** In an early study of 956 patients with acute IS,  
43 determinants of long-term mortality were age, obesity, cardiac arrhythmias, diabetes mellitus,  
44 coronary heart disease and organic brain syndrome at discharge from hospital; interestingly,  
45 hypercholesterolemia and smoking did not affect long-term outcome<sup>28</sup>. More recent studies uniformly  
46 identified age and stroke severity, usually assessed on the NIHSS or similar scales, as biomarkers of  
47 long-term functional outcome and mortality after stroke<sup>29,30</sup>. Fibrinogen has been related to long-term  
48 outcome after stroke<sup>31,32</sup>. There have been conflicting data on the predictive value of serum bilirubin  
49 levels on the long term risk of cardiovascular disease. While some studies are in favor of a predictive  
50 value<sup>33-35</sup>, others are not<sup>36</sup>. Also, CRP levels have been reported to impact the functional long-term  
51 outcome after IS<sup>37</sup>, and early neurological deterioration after IS has been related to decreasing  
52 albumin levels, elevated CRP and fibrinogen levels<sup>38</sup>. Potential biomarkers for occult cancer in IS  
53 patients include elevated D-dimers, fibrinogen, and CRP; infarction in multiple vascular territories; and  
54 poor nutritional status<sup>39</sup>. Interestingly, IS patients with elevation of at least two of the following  
55 coagulation-related serum markers, that is, D-dimer, prothrombin fragment 1.2, thrombin-  
56 antithrombin complex and fibrin monomer, in the post-acute phase of stroke, were more likely to have  
57 occult cancer or recurrent stroke during follow-up for 1.4±0.8 years<sup>40</sup>. In another study of acute IS  
58 patients, high D-dimer levels at admission were independently associated with recurrent stroke and  
59  
60

1  
2  
3 all-cause mortality during follow-up for up to 3 years<sup>41</sup>. These findings underpin the idea of shared risk  
4 factors for unfavorable outcomes in IS as well as cancer and they suggest that there may be  
5 coagulation-related biomarkers indicating an early stage of carcinogenesis or stroke (see also Box 2).  
6 Nevertheless, the clinical biomarkers that currently exist for predicting outcome are limited in their  
7 performance and clinical utility, and there is a need to overcome the limitations of current predictive  
8 models<sup>42</sup>.  
9  
10

11 **Study Rationale and Aims.** The primary aim of the SASKit (“Senescence-Associated Systems  
12 diagnostics Kit for cancer and stroke”) study is to discover a set of molecular biomarkers for outcomes  
13 after PDAC and IS, which are specifically useful to predict disease-triggered deterioration of health  
14 (“disease deterioration” for short) in terms of probable sarcopenia<sup>43</sup>, reduced clinical performance  
15 and quality of life (QoL). The outcomes also include the (co-)morbidity of vascular events (here defined  
16 as stroke, myocardial infarction, and venous or arterial thromboembolism) in patients with PDAC,  
17 which are observed frequently apart from sarcopenia. Also included is the (co-)morbidity of any kind  
18 of cancer and of cognitive decline. Moreover, we consider mortality, as the most canonical outcome.  
19 Following up on the primary aim, we will investigate the nature of the molecular biomarkers to find  
20 out whether cellular senescence and other aging-associated processes are contributing to disease  
21 deterioration. As a secondary aim, we will search for potential *diagnostic* biomarkers related to cellular  
22 senescence and other aging-related processes that may differentiate healthy controls from PDAC or IS  
23 patients. Avoiding unclear or circular terminology, we define a biomarker in a very general fashion,  
24 simply as a feature (data point)  $f_1$  that successfully predicts another feature  $f_2$  at a later time-point<sup>44</sup>,  
25 in a biomedical context. Here, features may be composites, based on the measurement of individual  
26 features. Often, feature  $f_1$  refers to molecular data, while feature  $f_2$  refers to phenotypic data, such as  
27 clinical outcomes. Ultimately, we aim to identify biomarkers that are easy to measure, and that can  
28 then be validated in other studies to predict a clinically relevant outcome.  
29  
30  
31  
32  
33  
34

---

35 **Box 1: Aging and cellular senescence.** Extra lifetime gained over the last century led to the widespread  
36 emergence of age-related diseases that are rarely seen in younger people. Older patients are thus  
37 more likely to display several comorbidities, making treatment difficult and expensive. Over the last  
38 years, strong evidence has accumulated that the presence of senescent cells (i.e. non-dividing but  
39 secretory, damaged, and metabolically active cells that escape apoptosis) is causally involved in  
40 diseases such as atherosclerosis, cancer, fibrosis, pancreatitis, osteoarthritis, Alzheimer disease and  
41 metabolic disorders<sup>45 46</sup>. Evidence that senescent cells are not only correlated with aging and diseases,  
42 but are also causally involved, comes from recent studies, which transplanted senescent cells from old  
43 into young mice<sup>47</sup>. This resulted in persistent functional impairment as well as spread of cellular  
44 senescence to host tissues. Another strong line of evidence comes from experiments that actually  
45 removed senescent cells from aged mice by senolytics<sup>47-49</sup>. In each case an increase in lifespan and a  
46 delay of typical age related diseases was observed. Most recently, the results of human pilot trials of  
47 putative senolytic treatments in case of idiopathic pulmonary fibrosis and osteoarthritis have been  
48 reported. One team<sup>50</sup> treated idiopathic pulmonary fibrosis patients with dasatinib and quercetin and  
49 demonstrated safety as well as notable improvements in some physical abilities. Furthermore, a  
50 human phase-1 study demonstrated that a senolytic compound, which was applied locally in patients  
51 with osteoarthritis of the knee, was safe and well-tolerated<sup>51</sup>. A clinically meaningful improvement in  
52 several measures, including pain, function, as well as modulation of certain senescence-associated  
53 secretory phenotype (SASP) factors and disease-related biomarkers was observed after a single dose.  
54  
55  
56  
57  
58  
59  
60



**Box 2: Cellular senescence and the comorbidity of cancer and vascular events.** Some cancers such as PDAC can trigger vascular events by hyper-coagulation, reflecting Trousseau's syndrome first reported 150 years ago<sup>9</sup>. In turn, strong associations between coagulation, cellular senescence and the SASP were recently demonstrated<sup>52 53</sup>. While cellular senescence can suppress PDAC and cancerous proliferation in general, it also triggers tumor progression by fostering inflammatory processes, including the SASP, while on the other hand, after ischemic stroke, it attenuates recovery<sup>54-58</sup>. For both diseases, causal influences can be traced back to molecular determinants: PAI-1 (also known as SERPINE1 and part of the SASP) is involved in cancer-triggered thromboembolism<sup>55 57</sup> and stroke recovery in animals<sup>59</sup>. Other proteins involved in cellular senescence, specifically inflammatory cytokines such as IL6, and the lesser known osteopontin and gelsolin, are also markers for both PDAC and stroke<sup>60-63</sup>. The cyclin-dependent kinase CDK5<sup>64</sup> is implicated in the progression of PDAC as well as in the recovery from stroke<sup>58 65</sup>. Moreover, apart from being genetic risk factors<sup>66 67</sup>, the most prominent drivers of cellular senescence (p16/CDKN2A and p21/CDKN1A) also promote PDAC progression<sup>68</sup> and endothelial embolic and arteriosclerotic mechanisms of stroke<sup>69</sup>. Finally, two small-molecule interventions into cellular senescence, fisetin and quercetin, are both potential therapeutic agents of PDAC and stroke. In case of stroke, the blood-brain-barrier is passed by quercetin which improves stroke outcome<sup>70</sup>. In case of PDAC it was observed that quercetin inhibits pancreatic cancer growth *in-vitro* and *in-vivo*<sup>71</sup>. Fisetin is found in various fruits (especially strawberries) and it is chemically similar to quercetin, with strong putative senolytic effects, extending lifespan of mice even when intervention with fisetin started only at an advanced age<sup>72</sup>. In a study involving nude mice implanted with prostate cancer cells, treatment with fisetin significantly retarded tumor growth<sup>73</sup>. Also, in case of lung cancer, there is evidence for the beneficial effects of fisetin. One study showed that fisetin provides protection against benzo(a)pyrene [B(a)P]-induced lung carcinogenesis in albino mice<sup>74</sup> and another *in vivo* study demonstrated the synergistic effects of fisetin and cyclophosphamide in reducing the growth of lung carcinoma in mice<sup>75</sup>. Several other studies have also demonstrated its anticarcinogenic, neurotrophic and anti-inflammatory effects that are beneficial in numerous diseases, including pancreatic cancer and stroke<sup>76</sup>.

---

## Methods

The presentation is based on the reporting recommendations for tumor marker prognostic studies (REMARK), that is, items (1) – (11) of the REMARK checklist<sup>77</sup>. The study design is illustrated in Figure 1, while the data analysis plan is summarized in Figure 2.

### Study design

The SASKit ("Senescence-Associated Systems diagnostics Kit for cancer and stroke") study is designed as a prospective, observational, cohort study to identify biomarkers for disease deterioration in patients with PDAC or with IS and, specifically, for the (co-)morbidity of these diseases including vascular events and sarcopenia following the diagnosis of PDAC as well as cancer and cognitive decline following IS. All patients will be treated for their diseases in accordance with current guidelines or therapy standards and at the physician's discretion. Due to the observational study design, regular treatment of the patient is not affected apart from sampling blood (20 to 80 ml at up to 7 time-points over the next years). Assessment of disease deterioration will be based on standardized clinical performance measurements, and patient reported outcomes based on questionnaires (see below for details). Additionally, data from clinical charts and information from the general practitioner will be collected. The SASKit study is divided into two subtrials with a common control group, both featuring essentially the same outcomes, predictor measurements and data analysis approaches.

## Patient and Public Involvement

It was not possible to involve patients or the public in the design of the study.

## Characteristics of participants (patients and controls)

In the first subtrial (PDAC-subtrial), patients with an initial diagnosis of PDAC in locally advanced or metastatic stage without previous systemic therapy will be considered for enrolment, whereas patients with a (thromboembolic) IS of the supratentorial brain region within the past 3 to 10 days, with a definitive brain infarction volume >10 ml in an assessment by magnetic resonance imaging (MRI) will be considered for the second subtrial (IS-subtrial). Except for some explorative analyses, the subtrials will be analyzed separately.

Within both subtrials, eligible as controls are those without PDAC or IS and with no other malignant disease or other (hemorrhagic) stroke during the past two years. Potential controls will be recruited from persons who have lived in the same household as the patient within the last 2 years, have a maximum age difference of 12 years and are neither brothers nor sisters (i.e. spouses, second-degree relatives or friends). The controls are selected so that the age and gender structure approximately reflects the age and gender distribution of the patients. Therefore, the age and gender of the patients will be continuously recorded, and the controls selected in such a way that their frequency distribution of gender at any time corresponds approximately to that of the currently recruited patients.

The following criteria lead to exclusion from participation in the study for both patients and controls, *at time of recruitment*:

- previous or current medical tumor therapy
- other cancer within the past 2 years
- previous stroke with persistent deficit
- myocardial infarction within the past 2 years
- therapeutic anticoagulation within the past 2 years for longer than 1 month
- pre-existing dementia
- chronic heart failure stage NYHA IV
- terminal renal insufficiency with hemodialysis
- known HIV infection
- known active hepatitis C
- pregnancy
- age < 18 years.

Both subtrials will be implemented according to the same standardized protocol. After written informed consent of each participant, patients will be followed up at 3, 12, 24, 36 and 48 months after their inclusion in the trial, whenever possible. The PDAC-subtrial includes an additional time-point for examinations at 6 months after inclusion, given that mortality due to PDAC is expected to be accelerated as compared to IS. Controls will be followed up at 12, 24, 36, 48 months.

The study is expected to start in the second quarter of 2020 and will finish with the last participant's follow up at 48 months. Until that time, we expect that 50 PDAC patients, 50 IS patients, and 50 controls participated in the trial. The study will be conducted at the Rostock University Medical Center

(UMR), Germany at Clinic III - Hematology, Oncology, Palliative Medicine and at the Department of Neurology; the institutions of the other co-authors are supporting the study in a variety of ways. The study is registered at German Clinical Trials Register (DRKS00021184) and will be conducted following ICH-GCP.

### General health- and disease-related and demographic data

General data of the study participants will be recorded at the beginning of the study (“month 0”) and consist of the following: age, sex, BMI, temperature, blood pressure, heart rate (ECG). Furthermore, through interviews the following additional data will be recorded: vascular risk factors (arterial hypertension, diabetes, hyperlipidaemia, smoking habits), history of vascular events (stroke, myocardial infarction, venous or arterial thromboembolism), atrial fibrillation, history of cancer, current medication, surgery or blood transfusions in the past three months and vascular or cancerous events affecting any first-degree relatives. These data may provide influential factors for explorative analyses, or be employed to interpret and discuss the results of the study.

### Blood sampling

Blood sampling will be done in a standardized fashion, that is, fasting and between 8 and 10 am, for all assays. Routine blood parameters will be recorded at the time-points described above (months 0 to 48). These consist of differential blood count, reticulocytes, INR (International normalized ratio of prothrombin time), partial thromboplastin time, D-dimers, fibrinogen, factor XII, albumin, bilirubin, LDH, high-sensitive CRP, CA19-9, cholesterol, and HbA1c. Among the standard measurements, we also measure the liver parameters ALT, AST and AP as surrogate markers of liver disease.

Experimental blood analysis (PAI-1 and omics) will be done for patients at month 0 in case of PDAC, at month 0 or at month 3 in case of stroke (where the 3-month time point is taken if it reflects a better state of the patient as described by the NIHSS) (“baseline”). It will furthermore be repeated at month 3 in the case of PDAC, and at month 12 in the case of stroke (“landmark”). For controls, the experimental blood analysis will be carried out at month 0 and at month 12, assuming that for these, data do not change much in the 3 months after baseline. The justification for taking the better clinical state in case of stroke is the maximization of differences with the month 12 follow-up data. In terms of practicality (being able to calculate a biomarker signature sooner), however, the state at month 0 should be selected for all stroke patients. Since the blood sample will be taken pre-processed and frozen at month 0 in all cases, we are in principle able to perform the experimental blood analysis for all stroke patients at month 0, and we can do this analysis in retrospect if deemed necessary. We also take blood of PDAC patients at month 12, to have the option to do an experimental blood analysis based on these samples, if deemed useful. In the following we will refer to the *baseline* time-point (month 0, or month 3 in cases of stroke patients that improved) and the *landmark* time-point (month 3 for PDAC patients and month 12 for stroke patients and controls). The experimental blood analysis is done earlier for PDAC because of high expected mortality within the first year.

The experimental blood analysis includes PAI-1 (see *Box 2*) as well as high-throughput (omics) analyses, that is, transcriptomics and proteomics analysis in T cells and proteomics of serum. T cells are of interest because these cells were reported to carry the strongest signal with respect to cellular senescence, based on the marker p16<sup>78</sup>. We intend to measure gelsolin and osteopontin as well, provided that sufficiently standardized assays become available in due time; the blood collected for this measurement shall otherwise be used to measure cytokines/chemokines such as IL6, IL8 and TNF $\alpha$ , which are part of the SASP, by ELISA assays. At time of writing, we do not yet have reliable estimates on the amount of blood cells still available for measuring protein expression, so an antibody-based protein array (in case of low amounts), or mass spectrometry (in case of sufficiently high amounts) will

1  
2  
3 be used alternatively. For the blood serum, we intend to use the same protein measurement method.  
4 In the default case of a protein array, we plan to use the novel but dedicated “Senescence Associated  
5 Secretory Phenotype (SASP) Antibody Sampler Kit” (consisting of approx. 10 SASP-related proteins  
6 being measured; Cell Signaling Technology) for both cellular and serum proteomics. Further  
7 exploratory molecular analyses not (yet) funded but permitted based on the ethics approval include  
8 the following: single-cell analyses of blood, methylation assays for calculating epigenetic clocks <sup>79</sup>,  
9 genetics by SNP array or whole-genome sequencing, and telomere length. A separate ethics approval  
10 was granted for an optional skin biopsy; skin microbiome analyses are planned as well. More  
11 specifically, participants have the option to provide a skin biopsy of 5 mm from an area that is not  
12 usually visible. We expect that about 30-50% of the participants will opt in. We keep the biopsy in  
13 culture for several days and divide it into several pieces. Using these, we measure biomarkers of  
14 cellular senescence (specifically, senescence-associated  $\beta$ -galactosidase, which cannot easily be  
15 measured in blood) and we treat some pieces with compounds that may affect cellular senescence,  
16 such as quercetin or fisetin. Moreover, we plan to sample the microbiome of the forehead using a  
17 standard swab. This is a very simple procedure, motivated by the claim that a competitive epigenetic  
18 aging clock can be based on such a sample <sup>80</sup>.

19  
20  
21  
22  
23 Blood sample processing for the experimental analysis will be performed according to standard  
24 operating procedures (SOP) at the research laboratory of Clinic III - Hematology, Oncology, Palliative  
25 Medicine. The procedures include flow cytometric control of the sampling quality including distribution  
26 of cell types and vitality as performed in routine diagnostics. Isolation of peripheral blood mononuclear  
27 cells (PBMCs) will also be performed following the SOP used by the laboratory in routine diagnostics.  
28 T Cell separation will be performed according to an established work flow based on magnetic bead  
29 purification via Miltenyi MACS following manufacturer’s instructions. T-Cell fraction purity as well as  
30 vitality will then be verified by flow cytometric analyses as described above. Nucleic acid isolation as  
31 well as protein isolation will be further performed according to the SOP of the research laboratory  
32 performed using column separation (Qiagen, Hilden Germany). RNA integrity values (RIN) will be  
33 analysed using an Agilent Scientific Instruments Bioanalyzer as instructed by the manufacturer. RIN  
34 values above 6 will qualify for RNAseq or Clariom D Array analyses; for RNAseq average reads per  
35 sample will be set at approx. 40 x 10e6.

### 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 [Clinical performance measurements and patient-reported outcomes](#)

At baseline and at each follow-up, handgrip strength (“grip strength” for short) is measured using a digital hand dynamometer (Jamar Plus). The test is performed while sitting comfortably, shoulder adducted, elbow placed on the tabletop and flexed to 90 degrees, with the forearm and wrist in a neutral position <sup>81</sup>. The highest value of three measurements of maximal isometric contraction of the dominant hand, or if paralyzed due to IS, contraction of the unaffected hand, is documented in kg. Further, the following clinical performance measurements are evaluated by the study physician or study nurse according to standard protocols: ECOG Performance Status (ECOG PS) <sup>82</sup>, modified Rankin Scale (mRS) <sup>83</sup>, Canadian Study on Health & Aging Clinical Frailty Scale (CSHA-CFS) <sup>84</sup>, NIH-Stroke Scale (NIHSS) <sup>85</sup>, Montreal Cognitive Assessment (MOCA) <sup>86</sup>. All raters are certified for the applicable scores (mRS, NIHSS, MOCA). Patient-reported outcomes (measured by questionnaires) are the following: EQ-5D-5L and EQ-VAS (generic evaluation of QoL in 5 domains and overall on a visual analog scale) <sup>87</sup>, HADS-D (evaluation of anxiety and depression) <sup>88</sup>, WHODAS 2.0 (WHO Disability Assessment Schedule) <sup>89</sup>, PASE (physical activity scale for the elderly) <sup>90</sup>, and, for patients with PDAC, FACIT-Pal (evaluating QoL with focus on palliative symptoms and needs) <sup>91 92</sup>. All questionnaires are administered following the suppliers’ instructions.

### [Follow up data](#)

1  
2  
3 Apart from the clinical and patient-reported outcomes, further follow-up data are BMI, temperature,  
4 blood pressure, heart rate (ECG), atrial fibrillation, current medication, tumor treatment, comorbidity  
5 (any vascular or cancer event), hospital admissions or palliative care. Additionally, based on clinical  
6 charts and information from the general practitioner, we will record medication, (co-)morbidity and  
7 mortality. Just like the general health- and disease-related and demographic data recorded at time of  
8 recruitment, these data may provide influential factors for explorative analyses, or be employed to  
9 interpret and discuss the results of the study.  
10  
11

## 12 Endpoints

13  
14 In both subtrials, the primary endpoint is a composite measure of “disease deterioration” defined as  
15 the *first* occurrence within a follow-up interval of at least one of the following.  
16

- 17 a. Sarcopenia, measured by grip strength less than 27 kg for males and less than 16 kg for females  
18 (according to the revised European consensus, EWGSOP2 <sup>43</sup>).
- 19 b. Deterioration of clinical performance, that is, of the ECOG PS by at least two points (PDAC-  
20 subtrial), or of the mRS by at least one point (IS-subtrial).
- 21 c. Deterioration of QoL, described as a reduction of the EQ-5D-5L by at least 0.07 in the index  
22 score, **and** deterioration of at least 7 points in the EQ-VAS (ranging from 0-100).  
23  
24

25  
26 Deterioration will be considered between baseline (month 0) and the respective landmark (follow-up)  
27 investigation. As described above, for patients with IS who have improved their condition (measured  
28 by NIHSS) within the first 3 months, this time point (month 3) will be used as a baseline instead. Item  
29 (a) is the deterioration from “no sarcopenia” to “probable sarcopenia” as defined by current consensus  
30 <sup>43</sup>. Grip strength has been widely used for assessing muscle strength, which is currently used as the  
31 most reliable measure of muscle function, loss of which indicating sarcopenia <sup>43</sup>. ECOG PS is established  
32 in describing the general condition of patients with cancer, whereas mRS is established in patients with  
33 stroke. Death is reflected by both scores as ECOG PS of 5 or mRS of 6, and it will always consider death  
34 from any cause. The EQ-5D-5L evaluates QoL in five dimensions (mobility, self-care, usual activity,  
35 pain/discomfort, and anxiety/depression), all relevant for patients with PDAC and IS. Furthermore, it  
36 is a generic score so that results will be comparable for different diseases (as recently described in  
37 patients with stroke <sup>93</sup> and for the general population <sup>94</sup>). Even though disease-specific scores might  
38 evaluate symptom burden in even more detail, the EQ-5D-5L was recently shown to be comparable to  
39 QoL scores developed specifically for pulmonary embolism and deep vein thrombosis (that is, PEmb-  
40 QoL, VEINES-QOL/Sym and PACT-Q2) in terms of acceptability, validity and responsiveness <sup>95</sup>. A clinical  
41 deterioration in EQ-5D-5L is described as a minimal important difference in the range from 0.07 to 0.09  
42 index points and in VAS from 7 to 10 points <sup>96</sup>, which is the basis for the definition of item (c). Controls  
43 reach their endpoint by the same definition as the subcohort for which they serve as control; in any  
44 integrative analysis of both subtrials, a deterioration of the mRS by at least one point will be used as  
45 the criterion (instead of ECOG PS), because stroke patients in general have a slower deterioration than  
46 PDAC patients, and controls naturally have the slowest expected deterioration.  
47  
48  
49  
50  
51

52 The primary composite endpoint and all secondary endpoints will be evaluated in a first analysis, based  
53 on data obtained until summer 2021, and in a second analysis, based on data obtained until summer  
54 2023, and in a third analysis at the end of the study. The second analysis may be delayed until data of  
55 90% of the study participants are available (at least including the month 12 follow-up) and it may then  
56 constitute the “main” analysis of the study. To address potential impacts of COVID-19 on the primary  
57 and secondary endpoints, the typical COVID-19 symptoms as well as confirmed diagnosis of COVID-19  
58 are recorded for all study participants at each study visit. In addition, at month 12 the presence of  
59 serum anti-SARS-CoV-2 IgG antibodies will be analysed.  
60



The following secondary endpoints will be evaluated:

- each component of the primary endpoint (separately);
- occurrence of disease-specific (co-)morbidity, as follows
  - new vascular events (stroke, myocardial infarction, venous or arterial thromboembolism), specifically in patients with PDAC;
  - new cancer, specifically in patients with IS;
  - probable sarcopenia (based on grip strength);
  - cognitive decline (deterioration of MOCA by 3 points from best value at baseline);
- frailty, defined as a CSHA-CFS level of 6, 7, or 8;
- all-cause mortality.

Further, a sum-score summarizing all measurements of phenotypic variables (grip strength, clinical performance measurements, comorbid events, mortality) will be considered as a surrogate for “aging”, normalizing all continuous-scaled components in order to obtain a common scale with an average of zero and standard deviation of one. The components of the sum-score will all be given equal weight.

### Predictors

While all phenotypic features (grip strength, clinical performance, patient reported outcomes, comorbid events, mortality) are contributing to the definition of endpoints (as dependent variables/parameters), all routine and experimental blood features (PAI-1, omics) are considered to be potential predictors; these are also called the independent variables/parameters. This delineation is justified by (a) the paradigm that (clinical) relevance is tied to high-level phenotypes describing health and survival, specifically including QoL<sup>44</sup>, and (b) the goal of developing a “senescence-associated systems diagnostics kit” that includes a careful selection of biomarkers contributing, as much as possible, also to molecular-mechanistic insights into PDAC, IS and their (co-)morbidity, which we hypothesize to be related to cellular senescence and aging. Age and gender will be included as mandatory covariates (also termed confounders, that is, predictors which we do not aim to explore, or which we wish to improve upon) in all statistical models. Further covariates are smoking, liver dysfunction or disease, the baseline NIHSS score in case of IS, as well as locally-advanced vs metastatic PDAC and modality of treatment in case of PDAC. As described, the successful predictors identified by our study, following the statistical analyses outlined below, are called biomarkers; we wish to stress that these are only *candidates* for the ultimate goal of *clinically validated biomarkers*; in particular, they still need to be validated in further studies (based, e.g., on other cohorts). A set of biomarkers is also called a biomarker signature.

### Blinding and pseudonymization

No blinding will be done during the study. However, the primary composite endpoint will be documented without subjective influence due to standardized definitions. Thus, detection bias will be kept at a minimal extent. Furthermore, information bias will be minimized as we will use simple measurements, which are applied in daily practice or are self-reported and easy to perform (e.g. EQ-5D-5L). The rigorous inclusion of all eligible patients within the recruitment period will help to minimize selection bias. All patient data are pseudonymized to all investigators except for the attending physician and study nurse. Since all major data analyses are based on known information about the outcomes (e.g., supervised machine learning with cross-validation), the data analysis will also be performed based on the pseudonymized data. Protection of personal and clinical data of all patients and controls will follow all relevant legal regulations.

### Sample size

No formal sample size calculation was performed a-priori for this observational study. The prevalence of PDAC combined with the requirement to complete the study within a reasonable timeframe implied a target of 50 patients per group (PDAC, IS and control group). Nevertheless, a power analysis revealed that a sample size of 50 patients will have 80% power to detect a significant difference by a non-parametric Wilcoxon statistic between an AUC of 0.75 for a particular biomarker signature compared to the null hypothesis value of 0.5 at a significance level of 5% under the assumption that about three times as many patients will reach the primary endpoint, compared to patients who will not reach the primary endpoint<sup>97</sup>.

### Data Analysis Plan

**General considerations:** The guiding criteria for biomarker identification in the SASKit study are the maximization of the predictive signal, clinical relevance/utility, biomedical/molecular/clinical interpretability, and practicality/cost. Given the relatively low number of participants in this in-depth study, to maximize the signal for the endpoints and predictors given as outlined above, we must aim to use all available information. Regarding endpoints, whenever possible, we thus wish to consider the (censored) time-to-event information inherent in the baseline and follow-up examinations, and in the mortality data. The primary endpoint was defined to integrate expected clinical utility and maximum signal. In defining the (secondary) endpoints, we considered an array of clinically relevant single endpoints as well as a sum-score of all phenotypic measurements; we hypothesize that the latter carries the largest amount of signal. Given the small sample, we cannot set aside an extra validation dataset. For the predictors considered to be covariates/confounders, please see the section on “Predictors”, above. The data analysis plan is summarized in Figure 2.

**Data quality assessment and cleaning:** The need for (and the amount of) data cleaning cannot easily be estimated beforehand; we plan to follow the MarkAGE guidelines<sup>98</sup> to deal with missing values, and to detect and rectify outliers and batch artefacts.

**Predictor/Feature integration:** Regarding predictors (features), we first need to remember that we measure at baseline (at months 0 or 3) and at one landmark (main follow-up, that is, at months 3 or 12). While use of baseline features is unrestricted, use of landmark features is, of course, restricted to prediction of outcomes after the landmark. Further, we need to handle the high dimensionality of the omics features. Here, upfront feature integration, e.g., by averaging measurements as described below, is considered preferable specifically for the high-dimensional omics data, for the following reasons.

- 1) A small feature space allows for an easier understanding and interpretation<sup>99</sup>.
- 2) Integrated features can be used as input for both the standard biostatistics and the standard machine learning parts of the analysis.
- 3) Use of few features is more time-tested than newer methods featuring the joint calculation of the prediction model and the selection of the features, albeit the latter are quite often claimed to be superior by their developers.
- 4) Naturally, feature integration avoids multicollinearity and overfitting, and multiple testing is less of an issue. This counters the “curse of dimensionality” and “de-noises” the data towards better prediction performance<sup>99 100</sup>.

- 1
- 2
- 3 5) Feature integration allows the handling of feature heterogeneity, which in our case refers to
- 4 routine blood measurements as well as various omics data types.
- 5
- 6 6) In the *explorative* analyses, systems biology modelling and the parallelogram approach are
- 7 both supposed to deliver further small sets of integrated, highly informative features, which
- 8 may, e.g., dominate systems behaviour, or which are believed to translate well from animal
- 9 models to humans.
- 10
- 11

12 While most features will be available for the baseline and the landmark time-point, utilizing baseline  
13 data is clinically more useful, simply because the prediction for the endpoint is available much earlier.  
14 Nevertheless, in the explorative analyses, we will investigate the predictive power of *changes* in  
15 feature measurements from baseline to landmark, given that such changes may be more informative  
16 about future disease deterioration (and other endpoints) than just baseline values.

17  
18  
19  
20 **Specific omics data feature integration:** Notably, we face a heterogeneous “multi-view” dataset,  
21 usually referred to as “multi-omics”. Our feature integration approach (see above) is also known as a  
22 “late integration” type of analysis, implying that measurements for different omics data types are  
23 reduced early on to activation scores for pathways or subnetworks that are then integrated at a “late”  
24 level. To calculate the activation scores for subnetworks, we use, by default, the  
25 ExprEssence/FocusHeuristics *linkscore*<sup>101 102</sup>, taking the links (gene/protein interactions) from a  
26 functional interaction network defaulting to STRING. Our experience with the *linkscore* motivates us  
27 to include this method as one of the approaches proposed for feature integration in the following,  
28 influencing the calculation of up to 10 features on which the standard biostatistics and machine  
29 learning shall be based. Specifically, we take the average expression measurement for all patients  
30 (as a list of expression values, one per gene) and the average for all controls (as a list of expression  
31 values, one per gene) to calculate a *linkscore* for each STRING interaction, and assemble a  
32 “condensed” network including all interactions with a *linkscore* in that percentile for which the 50  
33 highest-scoring interactions are shown. These interactions form subnetworks<sup>103</sup>. We then take the  
34 average *linkscore* for each subnetwork as the subnetwork activation score. Alternative methods  
35 such as *keypathwayminer* will be used in the exploratory analyses, see below. For the pathways (such  
36 as KEGG), we will calculate pathway activation scores using Gene Set Variation Analysis (GSVA)<sup>104</sup>. This  
37 method calculates pathway activation scores from expression data, is suited for use with microarray  
38 as well as RNAseq data and performed strongly in a recent benchmarking analysis<sup>105</sup>. The GSVA-based  
39 pathway activation scores can subsequently be compared between patients and controls in the same  
40 way as normal gene expression data, calculating, for each pathway, a fold-change of the pathway  
41 activation scores between patients and controls. Here, we average over all patients and over all  
42 controls, respectively, using the *limma* R package and adjusting for age and gender of the individual  
43 patient/control pathway activation. An example of this approach is given in the GSVA publication,  
44 where differential pathway activation was identified between acute lymphoblastic lymphoma and  
45 mixed-lineage lymphoma<sup>104</sup>. The major downside of feature integration may be information loss;  
46 subsequent statistical and machine-learning-based analyses receive only a tiny fraction of the amount  
47 of information that is available in total.

48 Gene expression data (transcriptomics) will be our preferred omics data type. Nevertheless, proteins  
49 are closer to the phenotype than transcripts, so we wish to not ignore these. Therefore, we prepare to  
50 deal with both kinds of proteome data that we may expect (see “Experimental blood analyses”, above),  
51 as follows.  
52  
53  
54  
55  
56  
57  
58  
59  
60

1. Large-scale data, likely based on mass spectrometry, in the order of hundreds or more proteins that can be identified and measured in all the conditions investigated.
2. Small-scale data, likely based on antibody arrays, in the order of ten proteins or less.

Except for the raw data preprocessing depending on the platform, once log-fold changes describing differential expression are established, we thus expect to handle the large-scale proteome data essentially the same as the transcriptomics data, and the small-scale proteome data similarly to the blood routine data, for cells and serum alike. Overall, the omics data are expected to come along three main coordinates, that is,

1. as blood cell transcriptomics and proteomics as well as serum proteomics;
2. longitudinal in time (for baseline and landmark); and
3. for PDAC, IS and control.

All coordinates can be exploited for differential analyses, even though the PDAC and IS data will be analyzed separately except for some integrative *explorative* analyses (see below). In the *explorative* analyses, the *longitudinal* transcriptomics of the patients and controls will also be analyzed together, see below. For the standard biostatistics and machine learning analyses, we plan to employ 5 approaches to feature integration, each yielding a shortlist of 5 integrated features, as follows.

- 1) **(5 features)** A first shortlist of features will consist of the following expert selection from the routine blood measurements (incl. PAI-1): *neutrophil-lymphocyte-ratio, fibrinogen, high-sensitive C-reactive protein, albumin* and *PAI-1*.
- 2) **(5 features)** For the cellular gene expression measurements, we use ExprEssence/FocusHeuristics (see above) to calculate *the top-5 subnetworks scoring highest*.
- 3) **(5 features)** Again for the cellular gene expression measurements, we use GSVA (see above) to calculate the top-5 most strongly changing pathways as features.
- 4) + 5) **(10 features)**
  - a) In case of dealing with large-scale serum proteomics data, we proceed as in (2) + (3);
  - b) In case of dealing with small-scale serum proteomics data, we proceed as follows:
    - i) if the number of features measured successfully is in the order of 10, we refrain from any processing;
    - ii) if the number of features is in the order of around 10-100, we select the 10 features with the smallest p-values indicating differences between the mean values of patient and control, based on a t-test.

For genomic features as per (2), the feature measurements for an individual patient or control will then be the average linkscores of the 5 selected subnetworks, contrasting each patient with average control data, and each control with average patient data. For genomic features as per (3), the feature measurements for each patient/control will be the GSVA scores of the 5 selected pathways. By construction, we expect the resulting features to reflect the up/downregulation of disease-related transcripts/proteins or pathways/subnetworks. Using the GSVA-based integrated features as input to the biostatistical analyses employing Cox proportional hazard models, we are in fact closely following the "Survival analysis in ovarian carcinoma" example as described in the GSVA publication<sup>104</sup>. Regarding the expert selection from the routine blood measurements, we are aware that some of these features may be considered to have an almost trivial relationship to outcome prediction for the diseases we study; e.g. fibrinogen may correlate strongly with the size of the stroke-damaged brain area and may thus be considered a covariate. However, to our knowledge, none of these features are validated clinical biomarkers, and it is quite possible that a combination of simple biomarkers is key to

1  
2  
3 the best possible prediction. We selected the *neutrophil-lymphocyte-ratio* specifically because it is  
4 cheap to measure; it is, however, like many other blood-based features, easily influenced by acute  
5 infection.  
6  
7

8 **Exploratory feature integration:** Apart from the FocusHeuristics/ExprEssence *linkscore*, we employ  
9 alternatives such as *keypathwayminer*<sup>106</sup>. Further, we calculate pathway activation scores for the  
10 following senescence-related KEGG pathways, which include PAI-1 (see the Introduction) but do not  
11 refer to a specific disease, as of February 2020: *Cellular senescence*, *HIF-1 signaling pathway*, *p53*  
12 *signaling pathway*, *Apelin signaling pathway*, *Hippo signaling pathway*, *Complement and coagulation*  
13 *cascades*. “Early integration” by, e.g., first averaging transcript and protein expression on a single-gene  
14 basis, is also planned.  
15  
16  
17

18 **Choice of data analysis methods for biomarker discovery:** We will consider two main approaches of  
19 data analysis, one motivated by statistical methods, the other by machine learning approaches. While  
20 this delineation may ultimately be meaningless, we consider that regression is the core ingredient of  
21 the former, while supervised learning characterizes the latter. We will apply standard methods (mostly  
22 in biostatistics) and explore novel approaches (mostly in machine learning; preserving signal implies a  
23 focus on *supervised* approaches in this case). Data analysis for biomarker *discovery* trials in a *clinical*  
24 setting is usually described with a biostatisticians’ mindset, who also developed methods to cope with  
25 the high dimensionality of omics data (see below). On the other hand, the challenges of omics data  
26 also spurred the recent publication of many methods adopting machine learning, which however did  
27 not yet make it into clinical trial analysis routine, but which we wish to test (see below). We will focus  
28 on methods readily available in SAS or as R packages. Notably, the correct choice of method depends  
29 in part on known unknowns such as the strength of the signal (incl. the amount of missing data) in the  
30 routine blood measurements and the omics.  
31  
32  
33  
34  
35  
36

37 **Prediction model quality measures:** Unlike intervention trials with their highly standardized aim of  
38 establishing a statistically significant superiority (or non-inferiority) of one intervention compared to  
39 another (or to standard of care), observational biomarker trials are a more recent development with  
40 fewer precisely quantified criteria of success, and a stronger need to consider the effect size: even if a  
41 biomarker signature enables a significant improvement in predicting an outcome, raising the accuracy  
42 of the prediction, say, from 70% to 75% may not be clinically meaningful, depending on prevalence of  
43 the condition to be predicted, the cost of the biomarker measurement, etc. We thus aim to identify  
44 biomarkers making a maximum of *difference* in prediction accuracy, if we are able to compare to  
45 established scores (see also below). For the biostatistics part, the concordance statistics (c-index) will  
46 be used as an overall measure of predictive accuracy, and time-dependent ROC curves and AUC will  
47 be used to summarize the predictive accuracy at different cut-off points in time. For the machine  
48 learning part, the cross-validated accuracy and AUC/c-index, following<sup>99</sup>, are used, and to take care of  
49 a potential Simpson’s paradox we will either analyse the data stratified by gender, or we will add such  
50 an analysis and check for consistency. More generally, to investigate the role of confounders (and, if  
51 necessary, to correct for these) in the machine learning part, we wish to use the permutation technique  
52 described<sup>107</sup>. We expect that we can identify a set of biomarkers that affords an accuracy of 75% or  
53 more or an AUC of 0.75 or more in correctly predicting the primary endpoint with a precision of +/-  
54 12%<sup>108</sup>. This estimate of precision is based on half the width of a 95% confidence interval (CI) for a  
55 probability of 75%, by extension of item 6 of the tables of Sorzano et al<sup>108</sup>, which shows precision up  
56 to a sample size of N=30.  
57  
58  
59  
60



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40

**Standard biostatistical analyses:** A Cox proportional hazards regression model adjusted for age and gender will be used to estimate the hazard ratio (HR) and corresponding 95% CI to predict the primary composite endpoint separately within the PDAC cohort and IS cohort. The 5 shortlists of 5 features (see above) will be providing the canonical predictors, analyzed together. For selection of the most important features that might be related to the primary endpoint we will use a procedure proposed by Sauerbrei et al.<sup>109</sup>, as follows. First, 100 bootstrap samples will be generated. Then, a multivariate Cox proportional hazards regression model with backward elimination with selection level of 0.05 will be fitted to each replication of the original data set. In a second step features with a relative selection frequency of 30% or less over all bootstrap samples will be eliminated. In a third step each feature  $X_i$  for which the hypothesis of independence in combination with a feature  $X_j$  can be rejected will be eliminated if  $X_i$  is less important when  $X_j$  is included in the model, or if it does not gain importance when  $X_i$  is excluded from the model. All remaining features will be included in the final model. Graphical and numerical methods will be performed to establish the validity of the proportionality assumption<sup>110</sup> in the final model. Results will be reported as p-values, HRs and corresponding 95%-CIs. A p-value of  $p \leq 0.05$  will be interpreted as indicating statistical significance. From the final model a risk score will be calculated by multiplying the individual feature measurement of a patient with the estimated regression coefficient of each feature. The c-index will be used as an overall measure of predictive accuracy of the resulting score, a time-dependent ROC curve and AUC will be used to summarize the predictive accuracy of the score at specific times. All secondary endpoints will be evaluated using the same approach as for the primary endpoint except for the sum-score used as a surrogate for "aging". For this endpoint, a linear mixed effects model with random intercept and spatial power covariance structure will be fitted to the data to estimate the progression of "aging". The covariance structure is chosen to reflect the unequal intervals of follow up investigations. Model assumptions and model fit will be checked by visual inspection of residuals, and influence diagnostics. Missing values will be taken into account by a likelihood-based approach within the framework of mixed linear models with the assumption that missing values occur at random. Results will be reported as p-value assessed at a level of significance of 5% accompanied by the value of the test statistic and degrees of freedom. In addition, 95% CIs for the progression (slope) will be provided.

41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51

**Additional exploratory biostatistical analyses:** Again, the primary composite endpoint as well as all secondary endpoints will be evaluated separately within the PDAC cohort and IS cohort of the respective sub-trials. In a first approach, univariate Cox proportional hazard models adjusted for age and gender will be calculated for each omics feature (R package *survival*) using a cut-off of 0.05 on the false discovery rate. In a second approach, all omics features will be simultaneously considered in a multivariate Cox model, adjusted for age and gender. Towards this aim, a component-wise likelihood-based boosting algorithm proposed by Binder and Schumacher 2008<sup>111</sup> (R package *CoxBoost*) will be used to develop a biomarker signature.

52  
53  
54  
55  
56  
57  
58  
59  
60

**Standard machine learning:** For the machine learning part, the primary outcome and all secondary outcomes give rise to an assignment of predictor/feature lists to survival times, one such list per study participant, for which biomarkers are then learned in a supervised fashion. As described, in the standard analyses, feature integration (see above) will precede the actual calculation of the model ("deep" learning approaches that take in "all" features are part of the *exploratory* analyses, see below). In the same way as the standard biostatistics analyses, the same 5 shortlists of 5 features each (see above) will be providing the canonical predictors, analyzed together. Exploiting time-to-event

1  
2  
3 information, we will employ random survival forests (RSF) as described by <sup>112</sup> with the following  
4 advantages.  
5

- 6  
7 1. RSF can now be considered a time-tested approach, and it was the subject of a recent  
8 extensive review <sup>68</sup> and of a systematic comparison with LASSO approaches in the case without  
9 feature selection (see item 7 of the tables of Pi *et al* <sup>113</sup> for its competitive performance which  
10 is not reflected in their abstract).
- 11  
12 2. RSF can also work on essentially all features, without a preceding feature integration/selection  
13 step, and then be compared, in the explorative machine learning analyses described below, to  
14 survival support vector machines (SSVM) and to a novel method Path2Surv that “conjointly”  
15 performs feature selection and model training, see <sup>99</sup>.
- 16  
17 3. RSF was recently compared to Cox-nnet <sup>114</sup>, a neural network approach which we consider as  
18 very promising for the *exploratory* part, see also below.
- 19  
20 4. RSF offers a considerable degree of interpretability, given that RSFs are derived from decision  
21 trees.
- 22  
23 5. RSF is considered “completely data driven and thus independent of model assumptions” and  
24 “in case of high dimensional data, limitations of univariate regression approaches such as  
25 overfitting, unreliable estimation of regression coefficients, inflated standard errors or  
26 convergence problems do not apply” <sup>68</sup>.

27  
28 In the machine learning part, we calculate accuracy and AUC/c-index using cross-validation to make  
29 the best use of our limited sample size, following the setup of <sup>99</sup> and <sup>113</sup> (who, however, set aside  
30 separate validation datasets), and we assess the features as biomarkers by ranking them by their  
31 variable importance score.  
32

33  
34 ***Additional exploratory machine learning:*** Apart from the more time-tested standard machine learning  
35 described above, we will also explore methods that were proposed recently, for which it is less  
36 straightforward to tell whether these methods are fit-for-purpose in our case, even though they are  
37 usually claimed to be superior by their developers based on some test/validation data sets. Specifically,  
38 as mentioned above, we expect to test Path2Surv and SSVM <sup>99</sup> as well as Cox-nnet <sup>114</sup> (without prior  
39 feature integration); the latter in particular promises a high degree of interpretability. We further  
40 explore CNet (employing the censored-data variant), for interpretable biomarkers. We also plan to  
41 employ the PASNet <sup>115</sup>, SurvivalNet <sup>116</sup> and SVRc <sup>73</sup> packages. The longitudinal transcriptomics of the  
42 patients and the controls may also be analyzed integratively based on the “optimal discovery  
43 procedure” <sup>117</sup>, considering, however, that landmark feature data can only be used to predict events  
44 after the landmark. Finally, we will map the differential omics data onto a human “healthspan pathway  
45 map” <sup>118</sup>, that is, a set of clusters/pathways based on health-related genetic data that we assembled  
46 recently.  
47  
48  
49  
50  
51

52  
53 ***Explorative systems biology modelling, explorative parallelogram approach and transfer learning:***  
54 As mentioned, systems biology modelling and parallelogram <sup>119</sup> <sup>120</sup> extrapolation are supposed to  
55 deliver small sets of highly informative features, by contributing features that are dominating model  
56 behaviour or that are shown to translate from the SASKit animal model data. Given the comparatively  
57 small number of study participants (but in-depth measurements), we also wish to explore “transfer  
58 learning”, which aims to utilize large amounts of public knowledge in the form of latent variables.  
59 Specifically, we plan to use, and wish to develop further, the Multiplier <sup>121</sup> approach motivated by the  
60

1  
2  
3 analysis of rare-disease data. Multiplier utilizes the RNASeq-based recount2 compendium, and apart  
4 from the functional network and pathway data that we use in the feature selection part, this  
5 compendium is expected to be a main source of biological knowledge that enters the calculations for  
6 biomarker discovery.  
7  
8

9  
10 **Miscellaneous exploratory approaches and discovery of diagnostic biomarkers:** We will also use  
11 unsupervised machine learning to generate descriptive multi-omics correlation networks, as they were  
12 most recently employed by <sup>122</sup>, there supplemented by linear mixed effects models using (un-  
13 )restricted maximum likelihood approaches; in this very recent biomarker discovery trial of similar  
14 design as ours, but with many more longitudinal omics measurement time-points than ours, we could  
15 not identify other biomarker discovery methods being used. If genetic data become available, we will  
16 include these in some analyses; specifically, we will investigate the added value of *expression*  
17 *quantitative trait loci* (eQTL) analyses. PDAC and IS data will be analyzed together in some integrative  
18 *exploratory* analyses. In that case, the occurrence of specific endpoints will be evaluated according to  
19 the group membership (PDAC or IS). This means that in addition to the biomarker signature, a group  
20 variable, indicating PDAC or IS patients, will be included in the analysis, to assess the difference in the  
21 progression of the respective endpoints between PDAC and IS patients. We also wish to compare PDAC  
22 and IS patient data to data of healthy controls (adjusted for age and gender) by means of logistic  
23 regression models with the aim of identifying candidate biomarkers for the diagnosis of the respective  
24 disease; we then specifically investigate the association of these diagnostic biomarker candidates with  
25 cellular senescence and other aging-related processes (see also the next paragraph).  
26  
27  
28  
29  
30

31 **Further analyses, and comparison with existing biomarkers and biomarker signatures:** Towards the  
32 end, we will investigate the overlap for the various biomarker identification approaches we employed,  
33 assuming that the most frequently found biomarkers may be the most robust and valid ones.  
34 Moreover, we will compare with existing biomarkers and signatures. Regarding the prediction of  
35 vascular events, we will specifically calculate the Khorana and related scores <sup>17</sup> for comparison, and  
36 report the difference in performance. Further, for all biomarkers we find, we will check their  
37 association with cellular senescence, by manual inspection, literature investigation, comparison to  
38 CellAge <sup>123</sup> and the SASP Atlas <sup>52</sup> or by formal enrichment analyses if the number of biomarkers is  
39 sufficiently large to do this in a meaningful way. Also, in a final step, we plan to identify and filter out  
40 the biomarkers that are volatile in the controls. In addition, a comparison of the biomarker profiles  
41 before and after the co-morbid event is aimed for. Finally, for publicly available data of other trials  
42 with a sufficient overlap with our predictors, we will use these as validation datasets.  
43  
44  
45  
46  
47

## 48 Discussion

### 49 Limitations

50  
51 Arguably, the most serious limitation of the SASKit study is the low number of participants. We  
52 mentioned above that in the 4-year-time-frame of the entire study, at the Rostock University Medical  
53 Center we cannot expect to recruit many more than the 50 PDAC patients to be included in this study;  
54 we could recruit more stroke patients and more controls, but given the call for proposals that allowed  
55 this exploratory (not confirmatory) study to be applied for and funded, we considered that within a  
56 limited budget, in-depth omics characterization, animal models (to be detailed in a follow up  
57 publication) and a comprehensive data analysis plan including systems biology modelling were  
58 important aspects of our study that we did not want to exclude.  
59  
60

1  
2  
3 The two most obvious risks to the main goal of finding good biomarkers for the primary outcome based  
4 on the standard data analysis are the following. First, we found it hard to estimate the distribution of  
5 events as defined by the primary outcome; we cannot exclude that too many events take place already  
6 at the start of the study, or until the first follow-up, specifically in the PDAC subtrial, limiting the  
7 amount of information available to the subsequent time-to-event analyses. Then again, had we  
8 defined the primary outcome more conservatively, there would have been a chance that not enough  
9 events happen before the end of the study. Second, we could not identify role-model publications  
10 reporting results of biomarker explorations that made use of machine learning methods, except for,  
11 to some extent, Schussler-Fiorenza et al <sup>122</sup>, so that we enter unknown territory to some degree. The  
12 two most obvious risks to our goal of investigating the role of cellular senescence in the (co-)morbidity  
13 of PDAC and IS could be an insufficient prevalence of co-morbid events, and the complex role of  
14 treatment in case of PDAC, where additional cellular senescence is most likely triggered by therapeutic  
15 intervention <sup>124</sup>. Then again, all molecular high-throughput analyses are essentially explorative and we  
16 are open to discovering biomarkers of disease that do *not* relate to any of our pre-specified  
17 hypotheses.  
18  
19  
20  
21

## 22 Implications

23 We designed the SASKit study to synergistically deliver upon multiple aims that we consider to be of  
24 relevance for specific disease prognosis and treatment as well as for primary, secondary and tertiary  
25 prevention. Employing clinical performance measurements and patient-reported outcomes, we aim  
26 for clinical relevance and we suggest that prognostic biomarker signatures for general health and QoL  
27 are perhaps more important than (progression-free) survival, although there is much more data about  
28 the latter. Moreover, good disease treatment options are still lacking for PDAC as well as for stroke,  
29 and the more we find cellular senescence implicated in disease deterioration, at least in a subgroup of  
30 patients with a specific biomarker signature, the more confidently we can suggest, and further explore,  
31 seno-therapeutic interventions for these two diseases.  
32  
33  
34

35 Notably, we are in the process of starting a parallel human study testing, in healthy elderly people,  
36 interventions into cellular senescence, based on *food* rich in seno-interventional compounds, and we  
37 expect that many aspects of the study design presented herein will be adopted in that parallel study.  
38 That study will also investigate aging- and senescence-related outcomes, and as such it can be seen as  
39 a test of a cautious yet potentially very effective approach to primary prevention; if the *diagnostic*  
40 biomarkers we find in the SASKit study relate to cellular senescence, this observation would constitute  
41 further evidence for (cautious) seno-interventions, moving towards a kind of universal approach of  
42 disease prevention by tackling fundamental aging-related processes (see Boxes 1 and 2).  
43  
44  
45

46 Secondary prevention, aiming to reduce the impact of a disease that has already occurred, can  
47 ultimately be supported by the SASKit study, if we can demonstrate, and (in follow up studies) confirm,  
48 a distinctive role of cellular senescence (and/or other aging-related processes such as  
49 inflammation/inflammaging <sup>125</sup>) in disease deterioration as defined here. Finally, evidence for tertiary  
50 prevention by seno-therapeutic intervention, aiming to attenuate the impact of an ongoing disease, is  
51 also an option based on how accurate, relevant and specific our biomarkers will be.  
52  
53

54 Last but not least, we expect that the in-depth molecular analyses that we wish to conduct will provide  
55 mechanistic insights into the etiology of the diseases we study here, which we just see as models for  
56 the investigation of the fundamental role of aging in general, and of cellular senescence in particular,  
57 in disease and dysfunction.  
58  
59  
60

## Ethics and dissemination

The study protocol has been approved by the ethics committee of the UMR (*Ethikkommission an der Medizinischen Fakultät der Universität Rostock, A2019-0174*). Results shall be published after completion of the study, following standard guidelines.

### Abbreviations:

|            |   |
|------------|---|
| ALT        | Alanine Aminotransferase  |
| AP         | Alkaline Phosphatase  |
| AST        | Aspartate Aminotransferase  |
| AUC        | Area Under the Curve  |
| BMI        | Body Mass Index   |
| CA19-9     | Carbohydrate Antigen  |
| CEA        | Carcinoembryonic antigen  |
| CI         | Confidence interval   |
| COVID-19   | Coronavirus disease 2019  |
| CRP        | C-reactive protein  |
| CSHA-CFS   | Canadian Study on Health & Aging Clinical Frailty Scale             |
| ECOG       | Eastern Cooperative Oncology Group                                  |
| EQ-5D-5L   | EuroQoL 5-Dimension 5-Level   |
| EQ-VAS     | EuroQol Visual Analogue Scale                                       |
| FACIT-Pal  | Functional Assessment of Chronic Illness Therapy-Palliative         |
| HADS-D     | Hospital Anxiety and Depression Scale - German Version              |
| HR         | Hazard ratio  |
| INR        | International normalized ratio                                      |
| IS         | Ischemic Stroke   |
| LDH        | Lactate dehydrogenase   |
| MOCA       | Montreal Cognitive Assessment                                       |
| mRS        | Modified Rankin Scale   |
| NIHSS      | NIH-Stroke Scale  |
| NYHA       | New York Heart Association  |
| PASE       | Physical activity scale of the elderly                              |
| PDAC       | Pancreatic Ductal Adenocarcinoma                                    |
| PS         | Performance status  |
| QoL        | Quality of Life   |
| ROC        | Receiver-Operator Characteristic                                    |
| RSF        | Random survival forests   |
| SARS-CoV-2 | Severe acute respiratory syndrome coronavirus 2                     |
| SASKit     | Senescence-Associated Systems diagnostics Kit for cancer and stroke |
| SASP       | Senescence Associated Secretory Phenotype                           |
| WHODAS     | WHO Disability Assessment Schedule                                  |

### Contributorship statement

Conception, writing and revision: Larissa Henze, Uwe Walter, Hugo Murua Escobar, Christian Junghanß, Robert Jaster, Rüdiger Köhling, Falko Lange, Ali Salehzadeh-Yazdi, Olaf Wolkenhauer, Mohamed Hamed, Israel Barrantes, Daniel Palmer, Steffen Möller, Axel Kowald, Nicole Heussen, Georg Fuellen.



1  
2  
3 Specific clinical considerations: Larissa Henze, Uwe Walter.

4  
5 Specific experimental considerations: Hugo Murua Escobar.

6  
7 Data analysis plan: Daniel Palmer, Nicole Heussen, Georg Fuellen.

8  
9 Acquisition of funding: Larissa Henze, Uwe Walter, Hugo Murua Escobar, Christian Junghanß, Robert  
10 Jaster, Rüdiger Köhling, Ali Salehzadeh-Yazdi, Olaf Wolkenhauer, Georg Fuellen.

11  
12 Project coordination: Axel Kowald, Georg Fuellen.

### 13 14 Conflict of Interest

15  
16 Dr. Walter reports personal fees from Ipsen Pharma, grants and personal fees from Merz Pharma,  
17 personal fees from Allergan, personal fees from Bristol-Myers Squibb, personal fees from Daiichi  
18 Sankyo, personal fees from Bayer Vital, personal fees from Boehringer Ingelheim, personal fees from  
19 Pfizer, personal fees from Thieme, and personal fees from Elsevier Press, all outside the submitted  
20 work. The other authors have nothing to disclose.

### 21 22 Funding

23  
24 We acknowledge the financial support by the Federal Ministry of Education and Research (BMBF) of  
25 Germany for the SASKit study (FKZ 01ZX1903A). The funder had no role in the design of the study.

### 26 27 Data sharing statement

28  
29 No data available.

### 30 31 References

- 32  
33 1. Collaborators GBDPC. The global, regional, and national burden of pancreatic cancer and its  
34 attributable risk factors in 195 countries and territories, 1990-2017: a systematic analysis for  
35 the Global Burden of Disease Study 2017. *Lancet Gastroenterol Hepatol* 2019;4(12):934-47.  
36 doi: 10.1016/S2468-1253(19)30347-4
- 37  
38 2. Kleeff J, Korc M, Apte M, et al. Pancreatic cancer. *Nat Rev Dis Primers* 2016;2:16022. doi:  
39 10.1038/nrdp.2016.22 [published Online First: 2016/05/10]
- 40  
41 3. Llop E, P EG, Duran A, et al. Glycoprotein biomarkers for the detection of pancreatic ductal  
42 adenocarcinoma. *World journal of gastroenterology* 2018;24(24):2537-54. doi:  
43 10.3748/wjg.v24.i24.2537
- 44  
45 4. Carrato A, Falcone A, Ducreux M, et al. A Systematic Review of the Burden of Pancreatic Cancer in  
46 Europe: Real-World Impact on Survival, Quality of Life and Costs. *Journal of gastrointestinal  
47 cancer* 2015;46(3):201-11. doi: 10.1007/s12029-015-9724-1
- 48  
49 5. Cruz-Jentoft AJ, Sayer AA. Sarcopenia. *Lancet* 2019;393(10191):2636-46. doi: 10.1016/S0140-  
50 6736(19)31138-9 [published Online First: 2019/06/07]
- 51  
52 6. Taieb J, Pointet AL, Van Laethem JL, et al. What treatment in 2017 for inoperable pancreatic cancers?  
53 *Annals of oncology : official journal of the European Society for Medical Oncology*  
54 2017;28(7):1473-83. doi: 10.1093/annonc/mdx174
- 55  
56 7. Menapace LA, Peterson DR, Berry A, et al. Symptomatic and incidental thromboembolism are both  
57 associated with mortality in pancreatic cancer. *Thrombosis and haemostasis* 2011;106(2):371-  
58 8. doi: 10.1160/TH10-12-0789
- 59  
60 8. Grilz E, Posch F, Konigsbrugge O, et al. Association of Platelet-to-Lymphocyte Ratio and Neutrophil-  
to-Lymphocyte Ratio with the Risk of Thromboembolism and Mortality in Patients with Cancer.  
*Thromb Haemost* 2018;118(11):1875-84. doi: 10.1055/s-0038-1673401 [published Online  
First: 2018/10/09]

- 1
  - 2
  - 3
  - 4
  - 5
  - 6
  - 7
  - 8
  - 9
  - 10
  - 11
  - 12
  - 13
  - 14
  - 15
  - 16
  - 17
  - 18
  - 19
  - 20
  - 21
  - 22
  - 23
  - 24
  - 25
  - 26
  - 27
  - 28
  - 29
  - 30
  - 31
  - 32
  - 33
  - 34
  - 35
  - 36
  - 37
  - 38
  - 39
  - 40
  - 41
  - 42
  - 43
  - 44
  - 45
  - 46
  - 47
  - 48
  - 49
  - 50
  - 51
  - 52
  - 53
  - 54
  - 55
  - 56
  - 57
  - 58
  - 59
  - 60
9. Bonnerot M, Humbertjean L, Mione G, et al. Cerebral ischemic events in patients with pancreatic cancer: A retrospective cohort study of 17 patients and a literature review. *Medicine* 2016;95(26):e4009. doi: 10.1097/MD.0000000000004009
10. Navi BB, Reiner AS, Kamel H, et al. Association between incident cancer and subsequent stroke. *Ann Neurol* 2015;77(2):291-300. doi: 10.1002/ana.24325
11. Varki A. Trousseau's syndrome: multiple definitions and multiple mechanisms. *Blood* 2007;110(6):1723-9. doi: 10.1182/blood-2006-10-053736
12. Grilz E, Marosi C, Konigsbrugge O, et al. Association of complete blood count parameters, d-dimer, and soluble P-selectin with risk of arterial thromboembolism in patients with cancer. *J Thromb Haemost* 2019;17(8):1335-44. doi: 10.1111/jth.14484 [published Online First: 2019/05/18]
13. Poiree S, Monnier-Cholley L, Tubiana JM, et al. Acute abdominal aortic thrombosis in cancer patients. *Abdom Imaging* 2004;29(4):511-3. doi: 10.1007/s00261-003-0144-5 [published Online First: 2004/03/17]
14. Schattner A, Klepfish A, Huszar M, et al. Two patients with arterial thromboembolism among 311 patients with adenocarcinoma of the pancreas. *Am J Med Sci* 2002;324(6):335-8. doi: 10.1097/00000441-200212000-00009 [published Online First: 2002/12/24]
15. Liu Z, Jin K, Guo M, et al. Prognostic Value of the CRP/Alb Ratio, a Novel Inflammation-Based Score in Pancreatic Cancer. *Ann Surg Oncol* 2017;24(2):561-68. doi: 10.1245/s10434-016-5579-3 [published Online First: 2016/09/22]
16. Haas M, Heinemann V, Kullmann F, et al. Prognostic value of CA 19-9, CEA, CRP, LDH and bilirubin levels in locally advanced and metastatic pancreatic cancer: results from a multicenter, pooled analysis of patients receiving palliative chemotherapy. *J Cancer Res Clin Oncol* 2013;139(4):681-9. doi: 10.1007/s00432-012-1371-3
17. van Es N, Di Nisio M, Cesarman G, et al. Comparison of risk prediction scores for venous thromboembolism in cancer patients: a prospective cohort study. *Haematologica* 2017;102(9):1494-501. doi: 10.3324/haematol.2017.169060 [published Online First: 2017/05/28]
18. Khorana AA, Kuderer NM, Culakova E, et al. Development and validation of a predictive model for chemotherapy-associated thrombosis. *Blood* 2008;111(10):4902-7. doi: 10.1182/blood-2007-10-116327 [published Online First: 2008/01/25]
19. Kruger S, Haas M, Burkl C, et al. Incidence, outcome and risk stratification tools for venous thromboembolism in advanced pancreatic cancer - A retrospective cohort study. *Thromb Res* 2017;157:9-15. doi: 10.1016/j.thromres.2017.06.021
20. Faille D, Bourrienne MC, de Raucourt E, et al. Biomarkers for the risk of thrombosis in pancreatic adenocarcinoma are related to cancer process. *Oncotarget* 2018;9(41):26453-65. doi: 10.18632/oncotarget.25458 [published Online First: 2018/06/15]
21. Stahmeyer J, Stubenrauch S, Geyer S, et al. The frequency and timing of recurrent stroke—an analysis of routine health insurance data. *Dtsch Arztebl Int* 2019;116:711-7.
22. Ryan AS, Ivey FM, Serra MC, et al. Sarcopenia and Physical Function in Middle-Aged and Older Stroke Survivors. *Arch Phys Med Rehabil* 2017;98(3):495-99. doi: 10.1016/j.apmr.2016.07.015 [published Online First: 2016/08/18]
23. Scherbakov N, von Haehling S, Anker SD, et al. Stroke induced Sarcopenia: muscle wasting and disability after stroke. *Int J Cardiol* 2013;170(2):89-94. doi: 10.1016/j.ijcard.2013.10.031 [published Online First: 2013/11/16]
24. Sanossian N, Djabiras C, Mack WJ, et al. Trends in cancer diagnoses among inpatients hospitalized with stroke. *J Stroke Cerebrovasc Dis* 2013;22(7):1146-50. doi: 10.1016/j.jstrokecerebrovasdis.2012.11.016 [published Online First: 2012/12/19]
25. Uemura J, Kimura K, Sibazaki K, et al. Acute stroke patients have occult malignancy more often than expected. *Eur Neurol* 2010;64(3):140-4. doi: 10.1159/000316764 [published Online First: 2010/07/30]

- 1
  - 2
  - 3
  - 4
  - 5
  - 6
  - 7
  - 8
  - 9
  - 10
  - 11
  - 12
  - 13
  - 14
  - 15
  - 16
  - 17
  - 18
  - 19
  - 20
  - 21
  - 22
  - 23
  - 24
  - 25
  - 26
  - 27
  - 28
  - 29
  - 30
  - 31
  - 32
  - 33
  - 34
  - 35
  - 36
  - 37
  - 38
  - 39
  - 40
  - 41
  - 42
  - 43
  - 44
  - 45
  - 46
  - 47
  - 48
  - 49
  - 50
  - 51
  - 52
  - 53
  - 54
  - 55
  - 56
  - 57
  - 58
  - 59
  - 60
26. Cocho D, Gendre J, Boltès A, et al. Predictors of occult cancer in acute ischemic stroke patients. *J Stroke Cerebrovasc Dis* 2015;24(6):1324-8. doi: 10.1016/j.jstrokecerebrovasdis.2015.02.006 [published Online First: 2015/04/18]
27. Selvik HA, Thomassen L, Bjerkreim AT, et al. Cancer-Associated Stroke: The Bergen NORSTROKE Study. *Cerebrovasc Dis Extra* 2015;5(3):107-13. doi: 10.1159/000440730 [published Online First: 2015/12/10]
28. Weitbrecht WU, Kirchhoff D. [Long-term prognosis of cerebral infarct in comparison with a normal population]. *Versicherungsmedizin* 1995;47(2):46-9. [published Online First: 1995/04/01]
29. Meyer S, Verheyden G, Brinkmann N, et al. Functional and motor outcome 5 years after stroke is equivalent to outcome at 2 months: follow-up of the collaborative evaluation of rehabilitation in stroke across Europe. *Stroke* 2015;46(6):1613-9. doi: 10.1161/STROKEAHA.115.009421 [published Online First: 2015/05/09]
30. Drozdowska BA, Singh S, Quinn TJ. Thinking About the Future: A Review of Prognostic Scales Used in Acute Stroke. *Front Neurol* 2019;10:274. doi: 10.3389/fneur.2019.00274 [published Online First: 2019/04/06]
31. Pedersen A, Stanne TM, Redfors P, et al. Fibrinogen concentrations predict long-term cognitive outcome in young ischemic stroke patients. *Res Pract Thromb Haemost* 2018;2(2):339-46. doi: 10.1002/rth2.12078 [published Online First: 2018/07/27]
32. Swarowska M, Polczak A, Pera J, et al. Hyperfibrinogenemia predicts long-term risk of death after ischemic stroke. *J Thromb Thrombolysis* 2014;38(4):517-21. doi: 10.1007/s11239-014-1122-1 [published Online First: 2014/08/12]
33. Perlstein TS, Pande RL, Creager MA, et al. Serum total bilirubin level, prevalent stroke, and stroke outcomes: NHANES 1999-2004. *Am J Med* 2008;121(9):781-88 e1. doi: 10.1016/j.amjmed.2008.03.045 [published Online First: 2008/08/30]
34. Choi Y, Lee SJ, Spiller W, et al. Causal Associations Between Serum Bilirubin Levels and Decreased Stroke Risk: A Two-Sample Mendelian Randomization Study. *Arterioscler Thromb Vasc Biol* 2020;40(2):437-45. doi: 10.1161/ATVBAHA.119.313055 [published Online First: 2019/12/06]
35. Zhong P, Wu D, Ye X, et al. Association of circulating total bilirubin level with ischemic stroke: a systemic review and meta-analysis of observational evidence. *Ann Transl Med* 2019;7(14):335. doi: 10.21037/atm.2019.06.71 [published Online First: 2019/09/03]
36. Jorgensen ME, Torp-Pedersen C, Finer N, et al. Association between serum bilirubin and cardiovascular disease in an overweight high risk population from the SCOUT trial. *Nutr Metab Cardiovasc Dis* 2014;24(6):656-62. doi: 10.1016/j.numecd.2013.12.009 [published Online First: 2014/02/19]
37. Wang L, Li Y, Wang C, et al. C-reactive Protein, Infection, and Outcome After Acute Ischemic Stroke: A Registry and Systematic Review. *Curr Neurovasc Res* 2019;16(5):405-15. doi: 10.2174/1567202616666191026122011 [published Online First: 2019/11/19]
38. Martin AJ, Price CI. A Systematic Review and Meta-Analysis of Molecular Biomarkers Associated with Early Neurological Deterioration Following Acute Stroke. *Cerebrovasc Dis* 2018;46(5-6):230-41. doi: 10.1159/000495572 [published Online First: 2018/12/06]
39. Navi BB, Iadecola C. Ischemic stroke in cancer patients: A review of an underappreciated pathology. *Ann Neurol* 2018;83(5):873-83. doi: 10.1002/ana.25227 [published Online First: 2018/04/11]
40. Ellis D, Rangaraju S, Duncan A, et al. Coagulation markers and echocardiography predict atrial fibrillation, malignancy or recurrent stroke after cryptogenic stroke. *Medicine (Baltimore)* 2018;97(51):e13830. doi: 10.1097/MD.00000000000013830 [published Online First: 2018/12/24]
41. Nezu T, Kitano T, Kubo S, et al. Impact of D-dimer levels for short-term or long-term outcomes in cryptogenic stroke patients. *J Neurol* 2018;265(3):628-36. doi: 10.1007/s00415-018-8742-x [published Online First: 2018/01/27]
42. Chaudhary D, Abedi V, Li J, et al. Clinical Risk Score for Predicting Recurrence Following a Cerebral Ischemic Event. *Front Neurol* 2019;10:1106. doi: 10.3389/fneur.2019.01106 [published Online First: 2019/11/30]

- 1
- 2
- 3
- 4 43. Cruz-Jentoft AJ, Bahat G, Bauer J, et al. Sarcopenia: revised European consensus on definition and
- 5 diagnosis. *Age Ageing* 2019;48(1):16-31. doi: 10.1093/ageing/afy169 [published Online First:
- 6 2018/10/13]
- 7 44. Fuellen G, Jansen L, Cohen AA, et al. Health and Aging: Unifying Concepts, Scores, Biomarkers and
- 8 Pathways. *Aging and Disease* 2019;10(4):883-900.
- 9 45. Yanai H, Fraifeld VE. The role of cellular senescence in aging through the prism of Koch-like criteria.
- 10 *Ageing research reviews* 2018;41:18-33. doi: 10.1016/j.arr.2017.10.004
- 11 46. Gonzalez-Meljem JM, Apps JR, Fraser HC, et al. Paracrine roles of cellular senescence in promoting
- 12 tumourigenesis. *British journal of cancer* 2018;118(10):1283-88. doi: 10.1038/s41416-018-
- 13 0066-1
- 14 47. Xu M, Pirtskhalava T, Farr JN, et al. Senolytics improve physical function and increase lifespan in
- 15 old age. *Nat Med* 2018;24(8):1246-56. doi: 10.1038/s41591-018-0092-9
- 16 48. Baker DJ, Childs BG, Durik M, et al. Naturally occurring p16(Ink4a)-positive cells shorten healthy
- 17 lifespan. *Nature* 2016;530(7589):184-9. doi: 10.1038/nature16932 [published Online First:
- 18 2016/02/04]
- 19 49. Baar MP, Brandt RMC, Putavet DA, et al. Targeted Apoptosis of Senescent Cells Restores Tissue
- 20 Homeostasis in Response to Chemotoxicity and Aging. *Cell* 2017;169(1):132-47 e16. doi:
- 21 10.1016/j.cell.2017.02.031 [published Online First: 2017/03/25]
- 22 50. Justice JN, Nambiar AM, Tchkonja T, et al. Senolytics in idiopathic pulmonary fibrosis: Results from
- 23 a first-in-human, open-label, pilot study. *EBioMedicine* 2019 doi:
- 24 10.1016/j.ebiom.2018.12.052
- 25 51. UNITY. UNITY Biotechnology Reports Promising Topline Data from Phase 1 First-in-human Study of
- 26 UBX0101 in Patients with Osteoarthritis of the Knee, 2019.
- 27 52. Tanaka T, Biancotto A, Moaddel R, et al. Plasma proteomic signature of age in healthy humans.
- 28 *Aging Cell* 2018;17(5):e12799. doi: 10.1111/accel.12799
- 29 53. Wiley CD, Liu S, Limbad C, et al. SILAC Analysis Reveals Increased Secretion of Hemostasis-Related
- 30 Factors by Senescent Cells. *Cell Rep* 2019;28(13):3329-37 e5. doi:
- 31 10.1016/j.celrep.2019.08.049 [published Online First: 2019/09/26]
- 32 54. Childs BG, Gluscevic M, Baker DJ, et al. Senescent cells: an emerging target for diseases of ageing.
- 33 *Nat Rev Drug Discov* 2017;16(10):718-35. doi: 10.1038/nrd.2017.116
- 34 55. Hisada Y, Mackman N. Cancer-associated pathways and biomarkers of venous thrombosis. *Blood*
- 35 2017;130(13):1499-506. doi: 10.1182/blood-2017-03-743211
- 36 56. Moir JA, White SA, Mann J. Arrested development and the great escape--the role of cellular
- 37 senescence in pancreatic cancer. *Int J Biochem Cell Biol* 2014;57:142-8. doi:
- 38 10.1016/j.biocel.2014.10.018
- 39 57. Valenzuela CA, Quintanilla R, Moore-Carrasco R, et al. The Potential Role of Senescence As a
- 40 Modulator of Platelets and Tumorigenesis. *Frontiers in oncology* 2017;7:188. doi:
- 41 10.3389/fonc.2017.00188
- 42 58. Posada-Duque RA, Barreto GE, Cardona-Gomez GP. Protection after stroke: cellular effectors of
- 43 neurovascular unit integrity. *Front Cell Neurosci* 2014;8:231. doi: 10.3389/fncel.2014.00231
- 44 59. Chan SL, Bishop N, Li Z, et al. Inhibition of PAI (Plasminogen Activator Inhibitor)-1 Improves Brain
- 45 Collateral Perfusion and Injury After Acute Ischemic Stroke in Aged Hypertensive Rats. *Stroke*
- 46 2018;49(8):1969-76. doi: 10.1161/STROKEAHA.118.022056 [published Online First:
- 47 2018/07/12]
- 48 60. Garcia-Berrocoso T, Penalba A, Boada C, et al. From brain to blood: New biomarkers for ischemic
- 49 stroke prognosis. *Journal of proteomics* 2013;94:138-48. doi: 10.1016/j.jprot.2013.09.005
- 50 61. Mendioroz M, Fernandez-Cadenas I, Rosell A, et al. Osteopontin predicts long-term functional
- 51 outcome among ischemic stroke patients. *Journal of neurology* 2011;258(3):486-93. doi:
- 52 10.1007/s00415-010-5785-z
- 53 62. Pan S, Chen R, Brand RE, et al. Multiplex targeted proteomic assay for biomarker detection in
- 54 plasma: a pancreatic cancer biomarker case study. *Journal of proteome research*
- 55 2012;11(3):1937-48. doi: 10.1021/pr201117w
- 56
- 57
- 58
- 59
- 60



- 1  
2  
3 63. Poruk KE, Firpo MA, Scaife CL, et al. Serum osteopontin and tissue inhibitor of metalloproteinase 1  
4 as diagnostic and prognostic biomarkers for pancreatic adenocarcinoma. *Pancreas*  
5 2013;42(2):193-7. doi: 10.1097/MPA.0b013e31825e354d  
6  
7 64. Alexander K, Yang HS, Hinds PW. Cellular senescence requires CDK5 repression of Rac1 activity.  
8 *Molecular and cellular biology* 2004;24(7):2808-19.  
9  
10 65. Feldmann G, Mishra A, Hong SM, et al. Inhibiting the cyclin-dependent kinase CDK5 blocks  
11 pancreatic cancer formation and progression through the suppression of Ras-Ral signaling.  
12 *Cancer Res* 2010;70(11):4460-9. doi: 10.1158/0008-5472.CAN-09-1107  
13  
14 66. Akinyemi R, Tiwari HK, Arnett DK, et al. APOL1, CDKN2A/CDKN2B, and HDAC9 polymorphisms and  
15 small vessel ischemic stroke. *Acta neurologica Scandinavica* 2018;137(1):133-41. doi:  
16 10.1111/ane.12847  
17  
18 67. Cremin C, Howard S, Le L, et al. CDKN2A founder mutation in pancreatic ductal adenocarcinoma  
19 patients without cutaneous features of Familial Atypical Multiple Mole Melanoma (FAMMM)  
20 syndrome. *Hereditary cancer in clinical practice* 2018;16:7. doi: 10.1186/s13053-018-0088-y  
21  
22 68. Wang T, Notta F, Navab R, et al. Senescent Carcinoma-Associated Fibroblasts Upregulate IL8 to  
23 Enhance Prometastatic Phenotypes. *Molecular cancer research : MCR* 2017;15(1):3-14. doi:  
24 10.1158/1541-7786.MCR-16-0192  
25  
26 69. Chen J, Huang X, Halicka D, et al. Contribution of p16INK4a and p21CIP1 pathways to induction of  
27 premature senescence of human endothelial cells: permissive role of p53. *American journal of*  
28 *physiology Heart and circulatory physiology* 2006;290(4):H1575-86. doi:  
29 10.1152/ajpheart.00364.2005  
30  
31 70. Tressera-Rimbau A, Arranz S, Eder M, et al. Dietary Polyphenols in the Prevention of Stroke.  
32 *Oxidative medicine and cellular longevity* 2017;2017:7467962. doi: 10.1155/2017/7467962  
33  
34 71. Angst E, Park JL, Moro A, et al. The flavonoid quercetin inhibits pancreatic cancer growth in vitro  
35 and in vivo. *Pancreas* 2013;42(2):223-9. doi: 10.1097/MPA.0b013e318264ccae  
36  
37 72. Yousefzadeh MJ, Zhu Y, McGowan SJ, et al. Fisetin is a senotherapeutic that extends health and  
38 lifespan. *EBioMedicine* 2018;36:18-28. doi: 10.1016/j.ebiom.2018.09.015  
39  
40 73. Khan FM, Zubek VB. Support Vector Regression for Censored Data (SVRC): A Novel Tool for Survival  
41 Analysis. Eighth IEEE International Conference on Data Mining. Pisa, Italy, 2008.  
42  
43 74. Ravichandran N, Suresh G, Ramesh B, et al. Fisetin, a novel flavonol attenuates benzo(a)pyrene-  
44 induced lung carcinogenesis in Swiss albino mice. *Food and chemical toxicology : an*  
45 *international journal published for the British Industrial Biological Research Association*  
46 2011;49(5):1141-7. doi: 10.1016/j.fct.2011.02.005  
47  
48 75. Touil YS, Seguin J, Scherman D, et al. Improved antiangiogenic and antitumour activity of the  
49 combination of the natural flavonoid fisetin and cyclophosphamide in Lewis lung carcinoma-  
50 bearing mice. *Cancer Chemother Pharmacol* 2011;68(2):445-55. doi: 10.1007/s00280-010-  
51 1505-8  
52  
53 76. Khan N, Syed DN, Ahmad N, et al. Fisetin: a dietary antioxidant for health promotion. *Antioxid*  
54 *Redox Signal* 2013;19(2):151-62. doi: 10.1089/ars.2012.4901  
55  
56 77. Altman DG, McShane LM, Sauerbrei W, et al. Reporting Recommendations for Tumor Marker  
57 Prognostic Studies (REMARK): explanation and elaboration. *PLoS Med* 2012;9(5):e1001216.  
58 doi: 10.1371/journal.pmed.1001216 [published Online First: 2012/06/08]  
59  
60 78. Liu Y, Sanoff HK, Cho H, et al. Expression of p16(INK4a) in peripheral blood T-cells is a biomarker of  
human aging. *Aging Cell* 2009;8(4):439-48. doi: 10.1111/j.1474-9726.2009.00489.x  
79. Ward-Caviness CK, Huffman JE, Everett K, et al. DNA methylation age is associated with an altered  
hemostatic profile in a multiethnic meta-analysis. *Blood* 2018;132(17):1842-50. doi:  
10.1182/blood-2018-02-831347  
80. Huang S, Haiminen N, Carrieri AP, et al. Human Skin, Oral, and Gut Microbiomes Predict  
Chronological Age. *mSystems* 2020;5(1) doi: 10.1128/mSystems.00630-19 [published Online  
First: 2020/02/13]



- 1
- 2
- 3
- 4 81. Sousa-Santos AR, Amaral TF. Differences in handgrip strength protocols to identify sarcopenia and
- 5 frailty - a systematic review. *BMC Geriatr* 2017;17(1):238. doi: 10.1186/s12877-017-0625-y
- 6 [published Online First: 2017/10/19]
- 7 82. Oken MM, Creech RH, Tormey DC, et al. Toxicity and response criteria of the Eastern Cooperative
- 8 Oncology Group. *Am J Clin Oncol* 1982;5(6):649-55. [published Online First: 1982/12/01]
- 9 83. van Swieten JC, Koudstaal PJ, Visser MC, et al. Interobserver agreement for the assessment of
- 10 handicap in stroke patients. *Stroke* 1988;19(5):604-7. doi: 10.1161/01.str.19.5.604 [published
- 11 Online First: 1988/05/01]
- 12 84. Rockwood K, Song X, MacKnight C, et al. A global clinical measure of fitness and frailty in elderly
- 13 people. *CMAJ* 2005;173(5):489-95. doi: 10.1503/cmaj.050051 [published Online First:
- 14 2005/09/01]
- 15 85. Lyden P, Brott T, Tilley B, et al. Improved reliability of the NIH Stroke Scale using video training.
- 16 NINDS TPA Stroke Study Group. *Stroke* 1994;25(11):2220-6. doi: 10.1161/01.str.25.11.2220
- 17 [published Online First: 1994/11/01]
- 18 86. Nasreddine ZS, Phillips NA, Bedirian V, et al. The Montreal Cognitive Assessment, MoCA: a brief
- 19 screening tool for mild cognitive impairment. *J Am Geriatr Soc* 2005;53(4):695-9. doi:
- 20 10.1111/j.1532-5415.2005.53221.x [published Online First: 2005/04/09]
- 21 87. Herdman M, Gudex C, Lloyd A, et al. Development and preliminary testing of the new five-level
- 22 version of EQ-5D (EQ-5D-5L). *Qual Life Res* 2011;20(10):1727-36. doi: 10.1007/s11136-011-
- 23 9903-x [published Online First: 2011/04/12]
- 24 88. Snaith RP, Zigmond AS. The hospital anxiety and depression scale. *Br Med J (Clin Res Ed)*
- 25 1986;292(6516):344. doi: 10.1136/bmj.292.6516.344 [published Online First: 1986/02/01]
- 26 89. Ustun TB, Chatterji S, Kostanjsek N, et al. Developing the World Health Organization Disability
- 27 Assessment Schedule 2.0. *Bulletin of the World Health Organization* 2010;88(11):815-23. doi:
- 28 10.2471/BLT.09.067231
- 29 90. Washburn RA, Smith KW, Jette AM, et al. The Physical Activity Scale for the Elderly (PASE):
- 30 development and evaluation. *J Clin Epidemiol* 1993;46(2):153-62. doi: 10.1016/0895-
- 31 4356(93)90053-4 [published Online First: 1993/02/01]
- 32 91. Lyons KD, Bakitas M, Hegel MT, et al. Reliability and validity of the Functional Assessment of Chronic
- 33 Illness Therapy-Palliative care (FACIT-Pal) scale. *J Pain Symptom Manage* 2009;37(1):23-32.
- 34 doi: 10.1016/j.jpainsymman.2007.12.015 [published Online First: 2008/05/28]
- 35 92. Sewtz C, Muscheites W, Kriesen U, et al. Questionnaires measuring quality of life and satisfaction
- 36 of patients and their relatives in a palliative care setting-German translation of FAMCARE-2
- 37 and the palliative care subscale of FACIT-Pal. *Ann Palliat Med* 2018;7(4):420-26. doi:
- 38 10.21037/apm.2018.03.17 [published Online First: 2018/06/05]
- 39 93. Golicki D, Niewada M, Karlinska A, et al. Comparing responsiveness of the EQ-5D-5L, EQ-5D-3L and
- 40 EQ VAS in stroke patients. *Qual Life Res* 2015;24(6):1555-63. doi: 10.1007/s11136-014-0873-7
- 41 [published Online First: 2014/11/27]
- 42 94. Ludwig K, Graf von der Schulenburg JM, Greiner W. German Value Set for the EQ-5D-5L.
- 43 *Pharmacoeconomics* 2018;36(6):663-74. doi: 10.1007/s40273-018-0615-8 [published Online
- 44 First: 2018/02/21]
- 45 95. Chuang LH, Cohen AT, Agnelli G, et al. Comparison of quality of life measurements: EQ-5D-5L versus
- 46 disease/treatment-specific measures in pulmonary embolism and deep vein thrombosis. *Qual*
- 47 *Life Res* 2019;28(5):1155-77. doi: 10.1007/s11136-018-2081-3 [published Online First:
- 48 2019/01/05]
- 49 96. Pickard AS, Neary MP, Cella D. Estimation of minimally important differences in EQ-5D utility and
- 50 VAS scores in cancer. *Health and quality of life outcomes* 2007;5:70. doi: 10.1186/1477-7525-
- 51 5-70
- 52 97. Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic
- 53 (ROC) curve. *Radiology* 1982;143(1):29-36. doi: 10.1148/radiology.143.1.7063747 [published
- 54 Online First: 1982/04/01]
- 55
- 56
- 57
- 58
- 59
- 60

- 1  
2  
3 98. Baur J, Moreno-Villanueva M, Kotter T, et al. MARK-AGE data management: Cleaning, exploration  
4 and visualization of data. *Mech Ageing Dev* 2015;151:38-44. doi: 10.1016/j.mad.2015.05.007  
5 [published Online First: 2015/05/26]  
6  
7 99. Dereli O, Oguz C, Gonen M. Path2Surv: Pathway/gene set-based survival analysis using multiple  
8 kernel learning. *Bioinformatics* 2019;35(24):5137-45. doi: 10.1093/bioinformatics/btz446  
9  
10 100. Buzdin A, Sorokin M, Garazha A, et al. Molecular pathway activation - New type of biomarkers for  
11 tumor morphology and personalized selection of target drugs. *Semin Cancer Biol* 2018;53:110-  
12 24. doi: 10.1016/j.semcancer.2018.06.003 [published Online First: 2018/06/24]  
13  
14 101. Warsow G, Greber B, Falk SS, et al. ExprEssence--revealing the essence of differential  
15 experimental data in the context of an interaction/regulation net-work. *BMC systems biology*  
16 2010;4:164. doi: 10.1186/1752-0509-4-164  
17  
18 102. Ernst M, Du Y, Warsow G, et al. FocusHeuristics - expression-data-driven network optimization  
19 and disease gene prediction. *Sci Rep* 2017;7:42638. doi: 10.1038/srep42638  
20  
21 103. Stahnke T, Gajda-Derylo B, Jünemann A, et al. Suppression of the TGF- $\beta$  pathway by a macrolide  
22 antibiotic decreases fibrotic responses by ocular fibroblasts in vitro. *Royal Society Open Science*  
23 2020;7(9):200441.  
24  
25 104. Hanzelmann S, Castelo R, Guinney J. GSEA: gene set variation analysis for microarray and RNA-  
26 seq data. *BMC Bioinformatics* 2013;14:7. doi: 10.1186/1471-2105-14-7  
27  
28 105. Geistlinger L, Csaba G, Santarelli M, et al. Toward a gold standard for benchmarking gene set  
29 enrichment analysis. *Brief Bioinform* 2020 doi: 10.1093/bib/bbz158 [published Online First:  
30 2020/02/07]  
31  
32 106. List M, Alcaraz N, Dissing-Hansen M, et al. KeyPathwayMinerWeb: online multi-omics network  
33 enrichment. *Nucleic Acids Res* 2016;44(W1):W98-W104. doi: 10.1093/nar/gkw373 [published  
34 Online First: 2016/05/07]  
35  
36 107. Neto E, Pratap A, Perumal T, et al. Using permutations to assess confounding in machine learning  
37 applications for digital health. *ArXiv* 2018; arXiv:1811.11920 or arXiv:1811.11920v1  
38  
39 108. Sorzano C, Tabas-Madrid D, Nunez F, et al. Sample Size for Pilot Studies and Precision Driven  
40 Experiments. *ArXiv* 2017; arXiv:1707.00222 or arXiv:1707.00222v2  
41  
42 109. Sauerbrei W, Schumacher M. A bootstrap resampling procedure for model building: application  
43 to the Cox regression model. *Stat Med* 1992;11(16):2093-109. doi: 10.1002/sim.4780111607  
44 [published Online First: 1992/12/01]  
45  
46 110. Lin DY. Cox regression analysis of multivariate failure time data: the marginal approach. *Stat Med*  
47 1994;13(21):2233-47. doi: 10.1002/sim.4780132105 [published Online First: 1994/11/15]  
48  
49 111. Binder H, Schumacher M. Allowing for mandatory covariates in boosting estimation of sparse  
50 high-dimensional survival models. *BMC Bioinformatics* 2008;9:14. doi: 10.1186/1471-2105-9-  
51 14 [published Online First: 2008/01/12]  
52  
53 112. Ishwaran H, Kogalur UB, Blackstone EH, et al. Random survival forests. *Ann Appl Stat*  
54 2008;2(3):841-60. doi: 10.1214/08-AOAS169  
55  
56 113. Pi L, Halabi S. Combined Performance of Screening and Variable Selection Methods in Ultra-High  
57 Dimensional Data in Predicting Time-To-Event Outcomes. *Diagn Progn Res* 2018;2 doi:  
58 10.1186/s41512-018-0043-4  
59  
60 114. Ching T, Zhu X, Garmire LX. Cox-nnet: An artificial neural network method for prognosis prediction  
of high-throughput omics data. *PLoS Comput Biol* 2018;14(4):e1006076. doi:  
10.1371/journal.pcbi.1006076 [published Online First: 2018/04/11]  
115. Hao J, Kim Y, Kim TK, et al. PASNet: pathway-associated sparse deep neural network for prognosis  
prediction from high-throughput data. *BMC Bioinformatics* 2018;19(1):510. doi:  
10.1186/s12859-018-2500-z  
116. Yousefi S, Amrollahi F, Amgad M, et al. Predicting clinical outcomes from large scale cancer  
genomic profiles with deep survival models. *Sci Rep* 2017;7(1):11707. doi: 10.1038/s41598-  
017-11817-6  
117. Bass A, Storey J. *bioRxiv* 2019 doi: 10.1101/571992

- 1  
2  
3 118. Moller S, Saul N, Cohen AA, et al. Healthspan pathway maps in *C. elegans* and humans highlight  
4 transcription, proliferation/biosynthesis and lipids. *Aging (Albany NY)* 2020;12(13):12534-81.  
5 doi: 10.18632/aging.103514 [published Online First: 2020/07/08]  
6  
7 119. Motwani HV, Frostne C, Tornqvist M. Parallelogram based approach for in vivo dose estimation  
8 of genotoxic metabolites in humans with relevance to reduction of animal experiments. *Sci*  
9 *Rep* 2017;7(1):17560. doi: 10.1038/s41598-017-17692-5  
10  
11 120. Kienhuis AS, van de Poll MC, Wortelboer H, et al. Parallelogram approach using rat-human in vitro  
12 and rat in vivo toxicogenomics predicts acetaminophen-induced hepatotoxicity in humans.  
13 *Toxicol Sci* 2009;107(2):544-52. doi: 10.1093/toxsci/kfn237  
14  
15 121. Taroni JN, Grayson PC, Hu Q, et al. MultiPLIER: A Transfer Learning Framework for Transcriptomics  
16 Reveals Systemic Features of Rare Disease. *Cell Syst* 2019;8(5):380-94 e4. doi:  
17 10.1016/j.cels.2019.04.003 [published Online First: 2019/05/24]  
18  
19 122. Schussler-Fiorenza Rose SM, Contrepolis K, Moneghetti KJ, et al. A longitudinal big data approach  
20 for precision health. *Nat Med* 2019;25(5):792-804. doi: 10.1038/s41591-019-0414-6  
21  
22 123. Avelar RA, Ortega JG, Tacutu R, et al. A Multidimensional Systems Biology Analysis of Cellular  
23 Senescence in Ageing and Disease. *bioRxiv* 2019  
24  
25 124. Demaria M, O'Leary MN, Chang J, et al. Cellular Senescence Promotes Adverse Effects of  
26 Chemotherapy and Cancer Relapse. *Cancer Discov* 2017;7(2):165-76. doi: 10.1158/2159-  
27 8290.CD-16-0241  
28  
29 125. Fulop T, Larbi A, Dupuis G, et al. Immunosenescence and Inflamm-Aging As Two Sides of the Same  
30 Coin: Friends or Foes? *Front Immunol* 2017;8:1960. doi: 10.3389/fimmu.2017.01960  
31

## Figure Legends

32  
33  
34 Figure 1: Study design of the SASKit study. Predictor and outcome measurements along the time axis  
35 are described.  
36

37  
38  
39 Figure 2: Data analysis plan of the SASKit study. Input, methods and output of the standard (but not  
40 the explorative) analyses based on biostatistics and machine learning are described in detail.  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

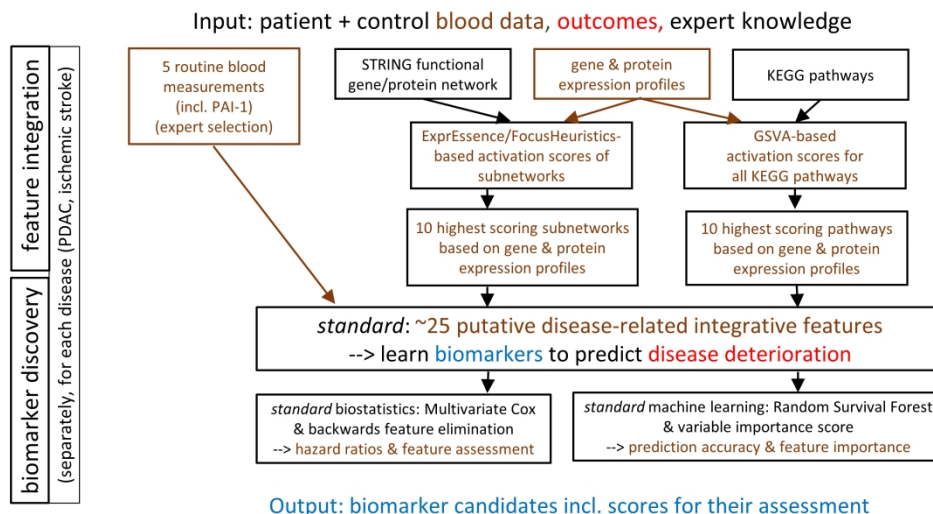
Patient + control, flowchart of activities

|  | month 0                | month 3          | month 6      | month 12   | month 24   | month 36   | month 48   |
|--|------------------------|------------------|--------------|------------|------------|------------|------------|
|  | (for all, by default:) | (patients only:) | (PDAC only:) | (for all:) | (for all:) | (for all:) | (for all:) |
| interview                                | ✓                      | ✓                | ✓            | ✓          | ✓          | ✓          | ✓          |
| general data, ECG                        | ✓                      | ✓                | ✓            | ✓          | ✓          | ✓          | ✓          |
| blood routine                            | ✓                      | ✓                | ✓            | ✓          | ✓          | ✓          | ✓          |
| incl. PAI-1                              |                        |                  |              |            |            |            |            |
| CA19-9 in patients                       | (✓)                    | (✓)              |              | (✓)        | (✓)        | (✓)        | (✓)        |
| collection T cells                       | ✓                      | ✓                |              | ✓          |            |            |            |
| collection serum                         | ✓                      | ✓                |              | ✓          |            |            |            |
| <b>grip strength</b>                     | ✓                      | ✓                | ✓            | ✓          | ✓          | ✓          | ✓          |
| <b>clinical performance measurements</b> | ✓                      | ✓                | ✓            | ✓          | ✓          | ✓          | ✓          |
| <b>patient-reported outcomes</b>         | ✓                      | ✓                | ✓            | ✓          | ✓          | ✓          | ✓          |
| <b>(FACIT-PAL: for PDAC)</b>             | (✓)                    | (✓)              | ✓            | (✓)        | (✓)        | (✓)        | (✓)        |

Note: T cells & sera are collected for omics to be thawed & analyzed as follows:  
 in case of PDAC only for month 0; and for month 3 (month 12 is rare),  
 in case of ischemic stroke only for either month 0 or month 3, i.e., for the better NIHSS score; and for month 12.

Study design of the SASKit study (human cohort; mouse studies designed to mirror the human study in part will be presented elsewhere). Predictor and outcome measurements along the time axis are described.

254x142mm (300 x 300 DPI)



Output: biomarker candidates incl. scores for their assessment

explorative: use other features/outcomes/methods; also investigate diseases jointly

23  
24  
25 Data analysis plan of the SASKit study (human cohort). Input, methods and output of the standard (but not  
26 the explorative) analyses based on biostatistics and machine learning are described in detail.

27  
28 254x142mm (300 x 300 DPI)