

Supplementary File:**The impact of molecular subtyping on pathological staging of Pancreatic Cancer**

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- North Shore Private Hospital Ethics Committee: Approval number NSPHEC 2016-016.
- Garvan Institute of Medical Research Approval number 1627.

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- Protocol number ICH-595, Humanitas Research Hospital, Milan

All data was transferred under the premise of approved data transfer agreements between the institutions and the University of Glasgow.

Supplementary Tables

Supplementary Table 1: Patient characteristics for the Glasgow, APCI, RNSH and Milan Cohorts

	Glasgow Cohort			APCI Cohort			Royal North Shore Cohort			Verona Cohort			Milan Cohort		
Variables	n = 366 No. (%)	Median DSS (months)	Pvalue (Log- rank)	n = 518 No. (%)	Median DSS (months)	Pvalue (Log- rank)	n = 283 No. (%)	Median DSS (months)	Pvalue (Log- rank)	n = 103 No. (%)	Median DSS (months)	P value (Log- rank)	n = 28 No. (%)	Median DSS (months)	Pvalue (Log- rank)
Sex															
Male	192 (52.5)	23.7	0.339	258 (49.8)	18.7	0.573	151 (53.4)	27.7	0.677	49 (47.6)	28.0	0.798	12 (42.8)	25.0	0.189
Female	174 (47.5)	25.7		260 (50.2)	21.0		132 (46.6)	34.1		54 (52.4)	30.0		16 (57.2)	12.0	
Age (years)															
Mean	63.0			66.7			67.1			66.01			70.32		
Median	64.3			68.0			68.4			68.00			72.50		
Range	30.8 – 86.0			28.0 – 88.0			33.9 – 91.3			37 – 87			43 – 85		
Outcome															
Follow-up (months)	3.2 – 166.1			18.0–163.8			11.1 – 98.9			7.0 – 93.0			6 – 55		
Median follow up(months)	41.0			47.0			33.0			56.5			17.5		
Death PDAC	261			394 (76.1)			147			64			15 (53.6)		
Death other	45			31 (6.0)			12			13			0 (0.0)		
Death Unknown	-			3 (0.6)			-			-			0 (0.0)		
Alive	60			89 (17.2)			123			26			13 (46.4)		
Lost to FU	-			1 (0.1)			-			0			0 (0.0)		
Stage (AJCC 8th)															
CPR*	5 (1.4)	NR	< 0.001	-		<0.001	6 (2.1)	NR	<0.001	0 (0.0)	-	0.033	0 (0.0)	N/A	0.150
IA	41 (11.2)	47.7		38 (7.3)	30.0		25 (8.8)	NR		4 (3.9)	55.0		1 (3.6)		
IB	36 (9.8)	26.7		94 (18.1)	19.6		46 (16.3)	34.9		7 (6.8)	32.0		4 (14.3)		
IIA	19 (5.2)	31.0		36 (6.9)	13.0		7 (2.5)	78.6		3 (2.9)	NR		2 (7.1)		
IIB	149 (40.7)	19.0		227 (43.8)	20.4		116 (41.0)	33.1		33 (32.0)	51.0		8 (28.6)		
III	116 (31.7)	22.4		108 (20.8)	17.0		83 (29.3)	23.9		56 (54.4)	22.0		13 (46.4)		
T Stage (AJCC 8th)															
CPR*	5 (1.4)	NR	<0.001			<0.001	6 (2.1)	NR	0.011	0 (0.0)	-	0.358	0 (0.0)		0.511
T1	81 (22.1)	39.0		101 (19.5)	34.4		48 (17.0)	NR		33 (32.0)	30.0		4 (14.3)	NR	
T2	207 (56.6)	22.6		292 (56.4)	18.6		175 (61.8)	30.5		56 (54.4)	28.0		13 (46.4)	14.0	
T3	73 (19.9)	13.5		119 (23.0)	13.2		53 (18.7)	27.6		14 (13.6)	14.0		11 (39.3)	19.0	
T4	0 (0.0)	-		2 (0.3)	-		1 (0.4)	-		0 (0.0)	-		0 (0.0)	-	
Unknown	0 (0.0)			4 (0.8)			0 (0.0)			0 (0.0)	-		0 (0.0)	-	
N Stage (AJCC 8th)															
N0	101 (27.6)	47.7	<0.001	169 (32.6)	22.2	<0.001	85 (30.0)	61.1	<0.001	14 (13.6)	55.0	0.007	7 (25.0)	NR	0.041
N1	149 (40.7)	19.0		228 (44.0)	20.3		116 (41.0)	33.1		33 (32.0)	51.0		8 (28.6)	NR	
N2	116 (31.7)	22.4		109 (21.0)	16.0		82 (29.0)	23.9		56 (54.4)	22.0		13 (46.4)	13.0	
Unknown	0 (0.0)			12 (2.3)			0 (0.0)			0 (0.0)			0 (0.0)		
Tumor Differentiation															
I / Well	30 (8.2)	36.3	<0.001	41 (7.9)	35.4	0.039	14 (4.9)	46.4	0.059	5 (4.8)	30.0	0.480	1(3.6)	NR	0.271
II / Moderate	229 (62.6)	25.0		340 (65.6)	21.0		186 (65.7)	34.1		62 (60.2)	30.0		6 (21.4)	NR	
III / Poor	100 (27.3)	17.1		130 (25.1)	17.0		76 (26.9)	26.5		35 (34.0)	19.0		21 (75.0)	17.0	
IV / undifferentiated	0 (0.0)	-		5 (1.0)	13.0		0 (0.0)	-		0 (0.0)	-		0 (0.0)	-	
Unknown	7 (1.9)	-		2 (0.4)			7 (2.5)			1 (1.0)	-		0 (0.0)		
Resection margin†															
Clear	119 (32.5)	36.7	<0.001	338 (65.3)	23.7	<0.001	123 (43.5)	54.2	0.001	59 (57.3)	48.0	0.002	12 (42.9)	NR	0.134
Involved	247 (67.5)	20.1		180 (15.4)	15.4		160 (56.5)	26.2		44 (42.7)	22.0		16 (57.1)	14.0	
Tumor Location															
Head / Uncinate	328 (89.6)	23.0	0.004	426 (82.2)	22.0	<0.001	268 (94.7)	30.2	0.055	87 (84.5)	29.0	0.848			
Body / Tail	38 (10.4)	39.0		92 (17.8)	12.1		15 (5.3)	40.2		16 (15.5)	23.0				

*CPR = complete pathological response; † = Margin status defined as original pathology report for each cohort (see methods)

Supplementary Table 2: The association of clinicopathological features and molecular subtype			
	Classical Pancreatic <i>n</i> = 330 (%)	Squamous <i>n</i> = 112 (%)	<i>P</i>-value (Chi-square)
T - stage			
T1	63 (19.1)	8 (7.3%)	0.013
T2	192 (58.4)	72 (65.5%)	
T3	74 (22.5)	30 (27.3%)	
N - stage			
N0	69 (21.0)	23 (20.7)	0.860
N1	121 (36.8)	38 (34.2)	
N2	139 (42.2)	50 (45.0)	
Tumor location			
Head	276 (88.7)	79 (76.7)	0.002
Body / Tail	35 (11.3)	24 (23.3)	
Grade / Differentiation			
I / II	230 (70.1)	47 (42.7)	<0.001
III / IV	98 (29.9)	63 (57.3)	
Perineural Invasion			
Negative	38 (11.7)	21 (19.1)	0.049
Positive	288 (88.3)	89 (80.9)	
Lymphovascular invasion			
Negative	168 (52.2)	45 (40.9)	0.041
Positive	154 (47.8)	65 (59.1)	
Margin R1 = 1mm			
Negative	92 (43.4)	26 (37.1)	0.358
Positive	120 (56.6)	44 (62.9)	
Margin R1 = 0mm			
Negative	193 (70.7)	61 (66.3)	0.428
Positive	80 (29.3)	31 (33.7)	

Supplementary Table 3: Molecular subtype cohort univariate survival analysis		
	Univariate Analysis	
	HR (95% CI)	<i>P</i>
Tumour Grade (High vs low)	1.57 (1.24 – 1.99)	<0.001
Margin Involvement (R1_{1mm})	2.03 (1.51 – 2.73)	<0.001
Lymphovascular Invasion (Positive)	1.88 (1.49 – 2.37)	<0.001
Perineural Invasion (Positive)	1.56 (1.09 – 2.22)	0.014
Lymph Node stage 8 th N1	1.36 (0.96 – 1.92)	0.082
Lymph Node stage 8 th N2	2.34 (1.89 – 3.26)	<0.001
T stage (8 th edition) T2	1.54 (1.09 – 2.17)	0.013
T stage (8 th edition) T3	2.29 (1.56 – 3.35)	<0.001
Adjuvant Chemotherapy	0.68 (0.51 – 0.90)	0.008
Tumor Location (Body/Tail vs Head)	1.45 (1.05 – 1.99)	0.023
Molecular Subtype (Squamous vs Classical)	1.98 (1.54 – 2.56)	<0.001

Supplementary Table 4: Molecular subtype cohort multivariate survival analysis		
	Multivariate Analysis	
	HR (95% CI)	<i>P</i>
<i>Step 1</i>		
T stage – T2	1.14 (0.62 – 2.11)	0.676
T3	1.58 (0.81 – 3.08)	0.179
Lymph Node – N1	1.14 (0.68 – 1.90)	0.632
N2	1.54 (0.89 – 2.65)	0.121
Grade (High grade)	1.61 (1.10 – 2.35)	0.015
Perineural Invasion	1.07 (0.63 – 1.82)	0.79
Lymphovascular Invasion	1.83 (1.24 – 2.71)	0.003
Tumor Location (body/tail)	1.83 (1.12 – 2.97)	0.015
Adjuvant Therapy	0.63 (0.44 – 0.90)	0.012
Margin (R1 = 1mm)	1.45 (0.96 – 2.20)	0.076
Molecular Subtype (Squamous)	1.50 (1.00 – 2.25)	0.051
<i>STEP 2</i>		
T stage – T2	1.14 (0.62 – 2.12)	0.672
T3	1.59 (0.81 – 3.09)	0.176
Lymph Node – N1	1.13 (0.68 – 1.90)	0.637
N2	1.54 (0.90 – 2.66)	0.119
Grade (High grade)	1.61 (1.10 – 2.35)	0.015
Lymphovascular Invasion	1.86 (1.29 – 2.69)	0.001
Tumor Location (body/tail)	1.83 (1.13 – 2.98)	0.015
Adjuvant Therapy	0.63 (0.44 – 0.91)	0.013
Margin (R1 = 1mm)	1.48 (1.00 – 2.19)	0.049
Molecular Subtype (Squamous)	1.49 (1.00 – 2.22)	0.053
<i>Step 3 – Final Model</i>		
Lymph Node – N1	1.21 (0.73 – 2.01)	0.47
N2	1.68 (0.98 – 2.86)	0.058
Grade (High grade)	1.66 (1.15 – 2.42)	0.007
Lymphovascular Invasion	1.85 (1.28 – 2.68)	0.001
Tumor Location (body/tail)	1.99 (1.24 – 3.22)	0.005
Adjuvant Therapy	0.63 (0.44 – 0.90)	0.010
Margin (R1 = 1mm)	1.50 (1.01 – 2.21)	0.044
Molecular Subtype (Squamous)	1.54 (1.04 – 2.28)	0.032

Supplementary Table 5: Classical subtype multivariate survival analysis		
<i>Final Model</i>		
	Univariate Analysis	
	HR (95% CI)	<i>P</i>
Tumor Grade (High vs low)	2.26 (1.44 – 3.54)	<0.001
Margin Involvement (R1_{1mm})	2.10 (1.40 – 3.17)	<0.001
Lymphovascular Invasion (Positive)	2.46 (1.62 – 3.73)	<0.001
T stage (8 th edition) T2	1.15 (0.61 – 2.16)	0.665
T stage (8 th edition) T3	2.23 (1.14 – 4.35)	0.019
Adjuvant Chemotherapy	0.62 (0.40 – 0.95)	0.028

Supplementary Table 6: Squamous subtype multivariate survival analysis		
<i>Final Model</i>		
	Univariate Analysis	
	HR (95% CI)	<i>P</i>
Perineural Invasion (Positive)	1.98 (0.89 – 4.40)	0.095
Tumor Location (Body/Tail vs Head)	2.46 (1.17 – 5.17)	0.018
Adjuvant Chemotherapy	0.55 (0.29 – 1.07)	0.079

Supplementary Table 7: The association of adjuvant therapy and molecular subtype (only showing for those with known accurate adjuvant data)			
	Classical Pancreatic <i>n</i> = 261 (%)	Squamous <i>n</i> = 82 (%)	<i>P</i> -value (Chi-square)
Adjuvant Chemotherapy			
Yes	194 (74.3)	51 (62.2)	0.025
No	67 (25.7)	31 (37.8)	
Chemotherapy Agent			0.396
Gemcitabine based	4 (3.5)	2 (6.3)	
Other	109 (96.5)	30 (93.8)	

Supplementary Table 8: The association of neoadjuvant therapy and molecular subtype (only showing for those who received neoadjuvant therapy)			
	Classical Pancreatic <i>n</i> = 13 (%)	Squamous <i>n</i> = 3 (%)	<i>P</i> -value (Chi-square)
Neoadjuvant Therapy			
FOLFIRINOX	0 (0.0)	1 (33.3)	0.376
Gemcitabine	4 (30.8)	0 (0.0)	
Gemcitabine / 5FU ChemoRad	3 (23.1)	1 (33.3)	
Gemcitabine / Abraxane	3 (23.1)	0 (0.0)	
Unknown	3 (23.1)	1 (33.3)	

Supplementary Figures

Figure Legends

Supplementary Figure 1: Kaplan-Meier survival curves for all patient cohorts stratified by (a – d) T-stage, (e – h) N-stage according to the AJCC 8th staging criteria and (i – l) resection margin status. The Verona and Milan cohorts were combined

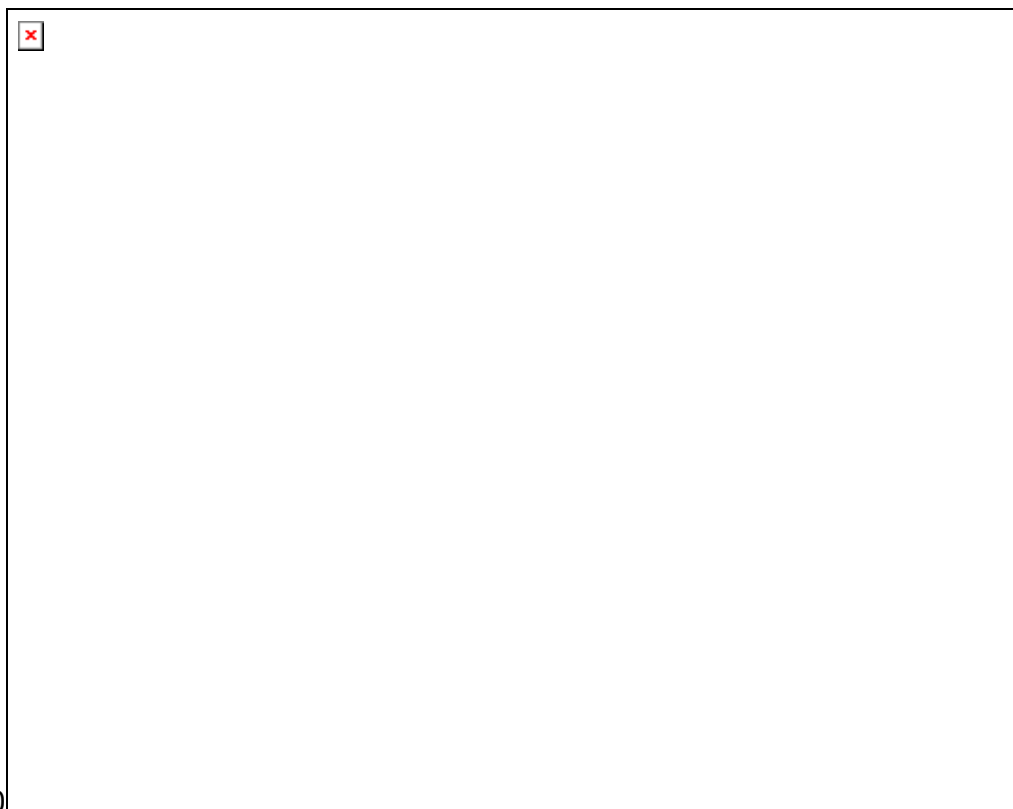
Supplementary Figure 2: Kaplan-Meier survival curves for all patients stratified by AJCC 8th staging criteria T-status (a - b), lymph node status (c - d) in both margin negative (a, c) and positive (b, d) patients.

Supplementary Figure 3: Kaplan-Meier survival curves for the molecular subtype cohort stratified by molecular subtype (squamous vs classical pancreatic) in both Margin negative (a) and margin positive (b) groups. Margin status defined as $R1_{\leq 1\text{mm}}$. Molecular subtype is prognostic in both margin positive and negative groups.

Supplementary Figure 4: Kaplan-Meier survival curves for the molecular subtype cohort stratified by lymph node involvement and margin status $R1_{0\text{mm}}$. Lymph node involvement is prognostic in (a) the entire cohort and (b) the Classical Pancreatic subtype, but not (c) in the Squamous subtype. Positive resection margin is prognostic in (d) the entire cohort and (e) the Classical Pancreatic subtype, but not in (f) the Squamous subtype.

Supplementary Figure 5: Kaplan-Meier survival curve for the molecular subtype cohort stratified by administration of adjuvant therapy. Irrespective of adjuvant therapy, squamous subtype had significantly worse prognosis.

Supplementary Figure 6: Kaplan-Meier survival curves for the molecular subtype stratified by S100A2 / A4 expression, lymph node status (a – c) and margin status (d-f). Margin status defined as $R1_{\leq 1\text{mm}}$. Biomarker status was determined using immunohistochemistry, and defined as both negative, either S100A2 or A4 positive or both biomarkers positive.



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Supplementary

Figure

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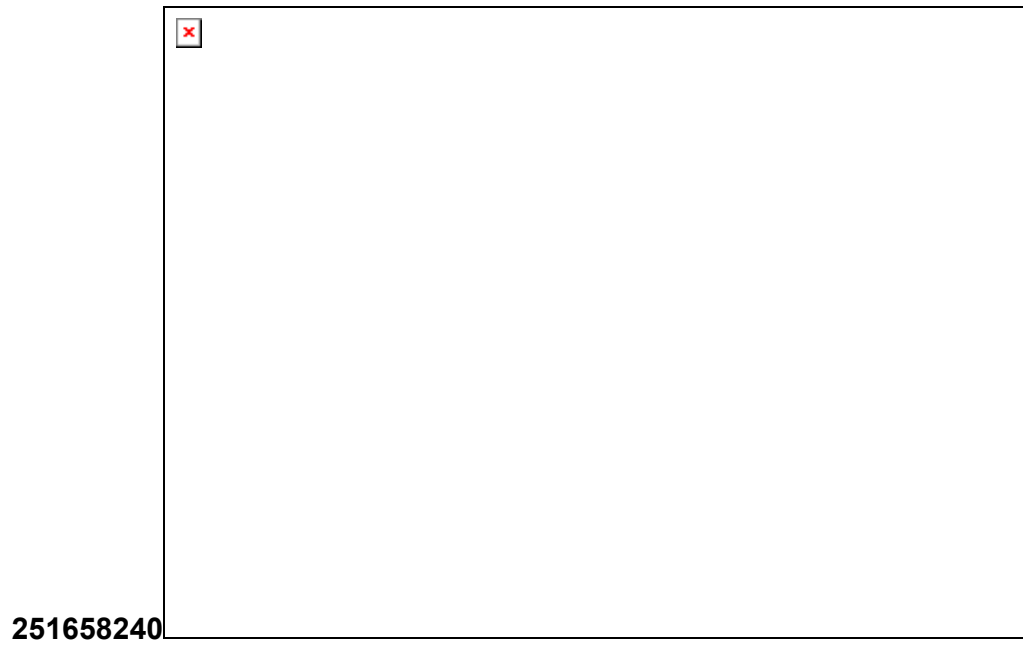


Supplementary Figure 2

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Supplementary Figure 3

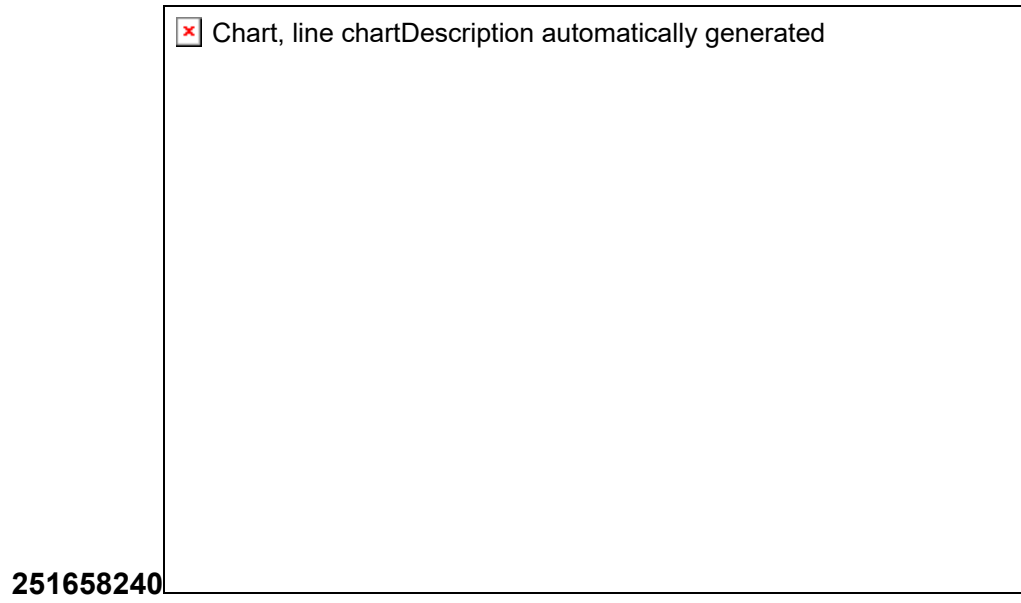


Supplementary Figure 4

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Supplementary Figure 5



Supplementary Figure 6