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OPINION PAPER

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How should clinical data be included in experimental studies of cancer immunology?

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Abstract Patients diagnosed with the same malignant disease are often heterogeneous with regard to age, complications, malignant cell morphology and tumor histology, disease stage, prognostic parameters, and previous therapy. Many of these factors can affect immunocompetent cells or influence the malignant cell susceptibility to immunotherapy. Summaries of relevant clinical information should therefore be included in cancer immunology studies to increase the present as well as the future scientific impact. Guidelines for selection of relevant information are suggested in the article.

Keywords Cancer · Clinical data · Immunology

Introduction

Studies of immunocompetent cells derived from cancer patients as well as characterization of the malignant cells will be an essential basis for the future designs of immunotherapeutic strategies in cancer treatment. However, the correct interpretation of such studies will often depend on the clinical context. In this article we discuss why it is important that authors, reviewers, and editors carefully consider how clinical data should be included/summarized in cancer immunology studies. We define clinical data as all available information from the routine handling of patients. This information includes (1) patient characteristics such as age, gender, ethnicity, general health conditions, previous disorders, and previous as well as current medical treatment (including anticancer therapy); (2) disease characteristics including tumor histology and clinical staging; and (3) cancer cell characteristics used as a part of the diagnostic or prognostic evaluation. Authors should carefully consider all these aspects when they select the relevant information to be included in their publications.

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Patient characteristics

Age and gender

The age (median and range) of included patients should be given. Most malignancies occur more frequently in elderly, and disease-induced alterations of the immune system may thereby be superimposed on age-dependent alterations [19]. Furthermore, an unexpected distribution of age and gender suggests that a study population represents a selected subset of patients (see below).

Ethnic differences

Certain malignancies show increased frequencies in certain geographic areas or certain populations, e.g., the high frequency of chronic lymphocytic leukemia in Europe and North America [8]. Furthermore, ethnic differences may in addition include different frequencies of genetic polymorphisms that involve (1) the responsiveness of the innate immune system against infectious agents, which depends on polymorphisms in immunoregulatory genes [9], and similar mechanisms may also be relevant for the reactivity against malignant cells; (2) cytotoxic drug metabolism and thereby treatment effects as well as toxicity [16]; (3) hormone-dependent carcinogenesis and metabolism of carcinogens [5, 11, 26]; (4) possibly immune reconstitution after chemotherapy and thereby susceptibility to treatment-related infections [17]; and (5) susceptibility of malignant cells to chemo-

therapy-induced and possibly also immune-induced apoptosis [2]. All these observations illustrate the importance of stating patient ethnicity in cancer immunology studies.

Patient selection

Very few clinical studies are population-based—i.e., all patients in the general population are included or patients are randomly selected. Many studies recruit their patients from selected groups referred to secondary or tertiary hospitals (e.g., mainly younger patients receiving the most intensive treatment, only elderly patients with good general health included, or patients selected based on the best response to initial treatment). Thus, authors have to consider whether their patients differ from the general patient population even when consecutive patients are included, and they should eventually describe briefly how patients have been selected.

Anticancer therapy

Anticancer treatment may interfere with immunological functions, and authors should generally summarize the previous and ongoing treatment of their patients, because (1) patients receiving various forms of intensive chemotherapy develop a period of severe treatment-induced leukopenia with blood leukocyte counts $< 0.5 \times 10^9$/l [27, 28]; (2) previous intensive chemotherapy can induce additional quantitative T-cell defects that persist for several months after hematopoietic reconstitution, as has been described for adult patients after autologous stem cell transplantation and after intensive chemotherapy for sarcomas, brain tumors, and hematological malignancies (Table 1) [7, 12, 13, 14, 15, 23, 24]; (3) certain chemotherapeutics may induce long-lasting quantitative T-cell defects even when initial leukopenia is avoided [8]; (4) therapy-induced quantitative defects may also occur for immunocompetent cells other than the T cells, but these defects have been less well studied [12]; and (5) the functional characteristics of circulating T cells may differ between patients with a similar quantitative T-cell defect [27, 28]. Thus, a summary of previous therapy is often required to discriminate between disease-associated and treatment-induced abnormalities of the immune system.

Table 1 Characterization of immune reconstitution after intensive conventional chemotherapy and high-dose chemotherapy followed by autologous stem cell transplantation [7, 12–15, 23, 24, 27, 28]

Rapid recovery of neutrophils for patients receiving conventional therapy and also for autografted patients when using peripheral blood mobilized progenitor cells.
Total numbers of circulating $CD4^+$ T cells often require several months before normal levels are reached (6–12 months), especially in elderly patients without postchemotherapy thymic hyperplasia. The reduced $CD4^+$ counts are due to a late reconstitution of thymus-dependent $CD4^+CD45RA^+$ T cells.
The reconstitution of thymus-dependent $CD8^+CD45RA^+$ T cells is also delayed and a gradual increase is observed during the 1st year after therapy.
A functional T-cell defect seems to persist for several months after therapy; this defect seems to be clinically important and predispose to complicating infections.

Disease characteristics

Disease staging, general health, and disease complications

Authors should describe the disease stage of their patients by using generally accepted classification systems. If the patients receive only palliative therapy, it should be clearly stated whether they have stable or progressive disease, eventually how stability was achieved, and how long it has lasted.

Patients with advanced malignancies develop cachexia, a clinical picture associated with altered immunological functions [1]. Cancer patients may in addition have reduced physical activity that causes additional immunological alterations [20], and they may be prone to specific complications that can affect immunocompetent cells—e.g., patients with gastrointestinal malignancies who develop malnutrition, biliary tract obstruction, or iron deficiency due to bleeding [6, 18]. Such complications are not necessarily reflected in the disease staging and can be observed before the development of cachexia. Authors should therefore consider whether additional information about specific complications should be included.

Microscopic architecture of the malignant disease

The histological diagnosis should be included, but additional morphological details may also be relevant. This can be exemplified by recent experimental studies describing an immunomodulatory effect of the stromal tissue in epithelial gastrointestinal cancers; the activated myofibroblasts in the stroma then seem to prevent penetration of monocytes and T cells into the tumor [10].

Studies of malignant cells

Studies of the malignant cells are important in cancer immunology because the cells may have immunomodulatory effects, e.g., through their cytokine release profile. Furthermore, the cancer cells' expression of surface molecules is important for the recognition by immunocompetent cells, and abnormalities in intracellular signaling pathways may determine the cells' susceptibility to immune-induced apoptosis. Such biological characteristics of the malignant cells are now used as predictive or prognostic factors in the evaluation of cancer patients [4, 8].

Cytotoxic drugs induce apoptosis, and adverse prognostic factors may thus reflect an intrinsic resistance to chemotherapy-induced proapoptotic signaling in the malignant cells. Induction of apoptosis is also important in T-cell cytotoxicity against target cells both through (1) the granule exocytosis mechanism involving the pore-forming perforin and the major effector enzyme granzyme B; and (2) oligomerization of death receptors with induction of apoptosis through the caspase cascade [22]. Thus, resistance against chemotherapy-induced apoptosis may reflect a general resistance that is relevant also for immune-induced apoptosis. This hypothesis is further supported by two recent observations describing associations between high-risk malignancies and expression of inhibitors of immune-induced apoptosis. Firstly, the serine protease inhibitor 9 can efficiently and irreversibly inactivate granzyme B, and inhibitor expression in non-Hodgkin's lymphomas is associated with high-grade malignancy [3]. Secondly, death receptor–induced apoptosis can be prevented by antiapoptotic molecules, exemplified by the cellular nonreceptor protein tyrosine phosphatase FAP-1 (Fas-associated phosphatase-1, also termed PTP-BAS, PTPL1, PTP1E, PTPN13) that is strongly expressed in pancreatic carcinomas (a cancer with a very low 2-year survival rate) and seems to protect against CD95-mediated apoptosis [25]. Taken together these data justify a relatively detailed summary of such cellular prognostic parameters for patients included in cancer immunology studies.

Concluding remarks

The above discussion strongly suggests that adequate clinical information has to be included in cancer immunology studies, and for the final presentation one has to consider how available space should be used for a summarized presentation of available and relevant information. Presentation of this information is necessary for interpretation of results, for comparison with previous and future studies, and to explain unexpected observations in exceptional patients. Adequate clinical information may thereby increase both the present as well as the future scientific impact of a study.

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