Patient-Derived Xenograft: An Adjuvant Technology for the Treatment of Metastatic Disease

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Key Words

Patient-derived xenograft · Metastatic cancer · Metastases · Personalized medicine · Pharmacological tool

Abstract

The occurrence of metastases severely affects prognosis for patients with cancer, making metastatic disease a daily societal challenge. Because of resistance to drugs, the potential curability with chemotherapy at the metastatic stage remains low. Large genomic analyses to identify new targets have their limitations due to intratumor heterogeneity when they are performed on tumor samples from primary tumors and because the functional value of molecular abnormalities in a cancer is usually not known. Additional tools are thus required for the development of new anticancer agents. The use of preclinical models is a key component of translational research in oncology. For four decades, xenograft models of human cancer cell lines injected subcutaneously in immunocompromised mice have been widely used, with disappointing results for predicting the clinical benefit of a new drug. Patient-derived xenografts are preclinical models rediscovered as innovative pharmacological tools, both for the pre-clinical development of anticancer drugs and as individual models for personalized treatment of metastatic disease.

Here, we review the recent progress reported using patient-derived xenografts for the treatment of metastatic disease, and discuss the feasibility of their implementation in daily oncological care.

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The occurrence of metastases severely affects prognosis for patients with cancer, making metastatic disease a daily societal challenge. The past 10 years have been dedicated to the development of new therapies targeting molecular abnormalities, with a few tremendous successes, such as the targeting of HER2 in a subpopulation of women with metastatic breast cancers with long-term survivors and potential curability with chemotherapy [1, 2], or the targeting of KIT in metastatic gastrointestinal stromal tumors [3–5]. However, because of resistance to drugs, the potential curability of chemotherapy at the metastatic stage remains anecdotic. In addition, large genomic analyses to identify new targets have their limitations.

One limitation is that most of these analyses have been performed on tumor samples from primary tumors. Pri-

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mary tumors are heterogeneous at the cellular and molecular levels [6–8], and metastases derive from selected aggressive clones, usually from tumor cells that have acquired resistance to first-line treatments, probably with few genomic variations between different metastases analyzed in a patient [9]. These tumor cells, which may be a minority in the primary tumor [10], are precisely those on which drug efficiency needs to be tested for individualized treatments of metastatic patients.

In order to avoid this limitation resulting from intra-tumor heterogeneity, multiple sampling of primary tumors should be done for molecular analyses. This is a precondition for adequate theranostic studies, but multiple sampling is difficult to implement in daily practice. Sampling from metastatic sites is even more difficult, but might be much more relevant.

Another limitation is the functional value of molecular abnormalities in a cancer is usually not known. For example, it took time to understand that epidermal growth factor receptor (EGFR) overexpression was not sufficient for anti-EGFR treatments to be efficient in metastatic colorectal cancers, and that it was also necessary for the tumor to be *K-RAS* wild type [11].

Additional tools are thus required for the development of new anticancer agents. The use of preclinical models is a key component of translational research in oncology. For four decades, xenograft models of human cancer cell lines injected subcutaneously in immunocompromised mice have been widely used, with disappointing results for predicting the clinical benefit of a new drug. This explains the growing interest for other models, such as patient-derived xenografts, which might better predict the clinical benefit of a drug. Several platforms of well-characterized patient-derived xenografts have emerged in recent years [12–14]. For metastatic disease, studies are ongoing to demonstrate their usefulness as preclinical pharmacological tools.

Murine Xenografts from Human Cancer Cell Lines Are Not Adequate Models for Preclinical Development of Anticancer Agents

Murine xenografts from human cancer cell lines offer some advantages: it is quite simple to establish a model and tumor volume is easy to measure when the graft is performed subcutaneously. For preclinical evaluation of an anticancer drug, xenografted mice can easily be synchronized. However, these models are very imperfect since most human cancer cell lines do not efficiently re-

flect human malignant tumors. One important explanation for this is their in vitro expansion for several months or years that results in genetic stresses and changes that do not reflect the carcinogenesis process in patient malignant tumors [15]. For example, no human glioblastoma cell line exhibits *EGFR* amplification although this is a frequent genomic abnormality in human glioblastomas [16]. Similarly, among the seven human prostate cancer cell lines that have been generated, only four express the androgen receptor and very few produce prostate-specific antigen [15], contrary to what is found in most prostate cancers in men.

For 40 years, murine xenografts derived from human cancer cell lines have been widely used for preclinical development of new anticancer agents. Until 1985, at the National Cancer Institute (NCI), the main model was a xenograft derived from human leukemia cell line P388, with poor efficiency in predicting the clinical benefit of a drug, particularly for nonhematological tumors [17]. A set of xenograft models derived from 60 human cancer cell lines has further been developed by the NCI to incorporate solid tumors in their platform for screening of new anticancer agents [18]. More than 10,000 compounds per year have been screened, but very few have yielded any clinical benefit [15]. In the early 2000s, Johnson et al. [19] assessed whether xenograft models derived from this panel could accurately predict the activity of new therapeutic agents in clinical trials. They compiled data for 39 drugs, selected on the basis of their cytotoxic activity in vitro on the panel of 60 lines. The 39 drugs were then tested on xenograft models with a median of 5 experiments and 4 different histological types. The correlation was low, leading to the conclusion that murine xenograft models derived from the NCI panel are not very reliable in predicting response to chemotherapy in patients.

Finally, xenografts from human cancer cell lines are highly controversial [15, 20–22], and their poor reliability explains the renewed interest in xenografts obtained from human tumor samples, also called ‘patient-derived xenografts’.

Patient-Derived Xenograft: A Rediscovered Relevant Pharmacological Tool

The concept of a patient-derived xenograft is not new, with the first report of a successful heterotransplantation of a human colon cancer in athymic nude mice in 1969 [23]. Twenty years ago, Klein et al. [24] established 6 models of patient-derived xenografts from prostatectomy

samples of 8 patients with prostate cancer. One model seemed particularly relevant since the tumor grafted was androgen dependent and had metastatic potential for the bone, thus reproducing the clinical and pathological aspects of the disease in patients.

For 20 years and mainly in the last decade, several institutions have established models of patient-derived xenografts, which are overall representative of the different types and subtypes of cancers, despite a larger representation of high-grade tumors [25–33].

For breast cancer, models from all tumor subtypes have been obtained, including an estrogen receptor-positive subtype with an engraftment rate under 1% [28, 34, 35]. Conversely, the triple negative subtype, the most aggressive subtype of breast cancer, is much better represented with an engraftment rate of over 35% [28, 34–38]. It is now clear that patient-derived xenografts more efficiently reflect tumor heterogeneity of cancers than xenografts from human cancer cell lines [20].

Patient-derived xenograft models also accurately reflect the known sensitivity profiles of given tumor types to drugs [26–28, 30, 32, 35]. Eighty-five patient-derived xenografts obtained from liver metastases of patients with colorectal cancers were treated with cetuximab, an anti-EGFR monoclonal antibody, versus placebo. In xenografts with *KRAS* wild-type status, cetuximab induced a 16.7% response rate [39], a result strikingly similar to the 13–22% response rate in patients treated with cetuximab or panitumumab [40–42]. Two other similarities with patient tumors were also observed in patient-derived xenografts [11, 43, 44]: (1) a higher response rate when the *EGFR* copy number was high, and (2) an absence of response to cetuximab when *KRAS* was mutated [39].

Xenografts obtained from fresh human tumor samples can be maintained and amplified by successive passages, without alteration of the genetic and biological characteristics of the tumor grown at the first passage [28, 31, 32, 36, 39].

These observations led to the suggestion of a more systematic implementation of patient-derived xenografts for the preclinical development of drugs [13]. Promising studies confirm that patient-derived xenografts might accurately predict the benefit of a drug or a combination of drugs in clinical practice [45–47]. Patient-derived pancreas cancer xenografts from the platform at Johns Hopkins enabled the prediction of the low benefit of temsirolimus, an mTOR inhibitor [45], and of AZD0530, a Src inhibitor [47], in the treatment of metastatic pancreas cancer. Another study combined a phase I-II study in patients with metastatic pancreatic cancer and the use of 11

patient-derived xenograft models to assess the benefit of the combination nab-paclitaxel + gemcitabine. Response rates were 48% in patients and 55% in xenograft models [46]. A further phase III study in metastatic pancreatic adenocarcinoma confirmed the benefit of this drug association, with a median survival of 8.5 months versus 6.7 months for the standard treatment with gemcitabine [48].

What Could Limit Their Use for Preclinical Development of Anticancer Drugs?

One major limitation is the low engraftment rate of patient-derived xenografts, usually under 20% for localized primary tumors grafted subcutaneously [28, 31, 33, 49]. Certain experimental conditions may significantly increase this rate: orthotopic grafting, grafting under the renal capsule [35, 50], or estradiol supplementation for breast cancer xenografts [35].

This low engraftment rate can be substantially higher in case of metastatic disease at the time of the graft, and even more so when metastatic tissue is grafted in immunodeficient mice [27, 29, 30, 51]: 80% for metastatic renal cell carcinomas (RCCs) [30] and for metastatic triple negative breast cancers [51]. For non-small-cell lung carcinoma, the engraftment rate of surgically removed brain metastases is 74% [27]. Similarly, in a series of 150 liver metastases from colorectal cancers systematically grafted in NOD/SCID mice, an engraftment rate of 87% was achieved [39]. Even at this advanced stage of the disease, engraftment in mice is predictive of shorter patient survival [27, 29].

In fact, tumor cells that metastasize in patients, which may be a minority in the primary tumor [10], are precisely those on which drug efficiency needs to be tested for individualized treatments of metastatic patients. These considerations should encourage the development of xenograft models from biopsies of metastases.

Another limitation could be the replacement of human tumor stroma in the primary grafted tumor by murine stroma after successive passages [34]. This raises a question about the benefit of drugs targeting human stromal cells, whether tumor vessels or inflammatory cells.

When analyzing human samples of RCC, our team recently demonstrated that sunitinib, a tyrosine kinase inhibitor with antiangiogenic effect, was able to generate resistance to its own therapeutic effect via induced hypoxia in cancer stem cells [52]. In human tumor samples obtained before and after treatment with sunitinib from the same patients with metastatic RCC, we observed that

renal cancer stem cells increased in numbers after treatment. Experimentally, we reproduced this effect of sunitinib in patient-derived xenograft models of human metastatic renal carcinomas.

Could Patient-Derived Xenografts Reflect Metastatic Disease?

The low engraftment rate of tumor samples from localized primary tumors could be linked to cellular heterogeneity in the primary tumors [28–31]. Only localized primary pancreatic cancers have a high engraftment rate (61%), but engraftment is associated with an increased risk of metastases and death [26]. Sixty-seven percent of these engrafted tumors have *Smad4* protein loss, a feature associated with a higher metastatic potential [26]. The engraftment rate of primary tumors is also higher in case of metastatic disease at diagnosis, up to 50% for RCCs [30].

Patient-derived xenografts could be a way to select the most aggressive clones in a primary tumor. Transcriptomic analyses performed on four primary localized pancreatic cancers and their corresponding xenografts at the fifth and tenth passages showed that the four tumor xenograft models had a metastatic gene expression signature [26]. Using whole-exome sequencing, Ding et al. [53] demonstrated great similarities between xenografts obtained from a primary breast cancer and its brain metastasis. Similar CGH array profiles were also found between a lymph node metastasis and xenografts obtained from the primary breast carcinoma, while the CGH array profile of the primary tumor was different [36].

While genomic concordance between metastases and xenografts is good, differences are observed between a primary tumor and a corresponding xenograft at its first passage. Indeed, when systematic molecular analyses are performed to compare the primary tumor with the grafted tumor, significant differences can be found [28, 31, 36, 39]. The number of genetic alterations is usually larger in the xenograft than in the primary tumor from which it derives [28, 36].

We established xenograft models of human primary RCCs, with an engraftment rate of 5% in case of localized disease, and of 36% in case of metastatic disease at diagnosis [31, 49]. For each xenograft model, we compared the primary tumor and the corresponding tumor xenografts at the first passage. Microsatellite analyses showed a 30% difference between a primary RCC and the corresponding xenograft at the first passage [31].

Assessing *TP53* abnormalities in multiple samples of 8 primary RCCs, xenografts derived from them, and metastatic samples, we found that tumor xenografts were more similar to the metastasis than to the primary RCCs they derived from.

The primary RCCs were heterogeneous with spatially separated subclones of p53-expressing cells, which we laser-microdissected. Assessing *TP53* mutations in these cells, we were able to track back to a minority subclone of *TP53*-mutated cells in the primary RCC, secondarily expanded in the corresponding lung metastasis, and two xenografts [49].

Could patient-derived xenografts reflect biological metastatic disease more than xenografts from cancer cell lines? This would not be a limitation, but rather an additional argument for their use, because in patients, chemotherapeutic agents primarily target metastatic disease, either micro- or macrometastatic.

Individual Patient-Derived Xenografts for Personalizing Treatment of Metastatic Cancers

Individual xenografts, which provide an innovative tool to amplify limited available quantities of human tissue, might thus reflect metastatic disease with good genetic stability after passages. This opens the field for individual use of patient-derived xenografts to predict sensitivity to chemotherapeutic agents.

The first reported case was a patient with metastatic pancreatic cancer treated with mitomycin after progression under a first-line treatment with gemcitabine. This patient was offered treatment with mitomycin, which had proved efficient on the xenograft model derived from his primary tumor. This treatment enabled an additional 22 months of disease control [54], while the theoretical median survival in metastatic pancreatic cancer is less than 9 months [55]. Encouraged by this result, the same team established 14 patient-derived xenograft models which were treated with an unselected panel of 63 different anticancer agents in combination or monotherapy. The authors reported excellent correlation between the antitumor activity of one drug in a xenograft model, and the antitumor effect of the same drug in the corresponding patient [56].

Another patient with a metastatic adenoid cystic carcinoma was enrolled in a phase I trial combining a pan-EGFR inhibitor and an anti-IGF1R monoclonal antibody because of the high antitumor effect of an IGF1R inhibitor on the patient's xenograft model obtained from a

brain metastasis sample. The treatment achieved control of rapidly growing liver metastases for at least 6 months, suggesting the possibility of implementing this strategy in certain phase I clinical trials [57].

In the light of these promising results, our team decided to combine the use of individual xenografts with genomic analyses of metastatic biopsy samples to personalize treatment for women with metastatic triple negative breast carcinomas. We postulated (1) that xenografts derived from metastatic samples would be more relevant than xenografts derived from primary tumors, and (2) and that even for this severe disease, the rapid engraftment (time lapse under 1.5 month) and a doubling time ranging from 4 to 17 days [28, 36] would allow for drug testing on xenografts while the woman was receiving first-line treatment. We first grafted two needle biopsy samples of metastases with an 80% engraftment rate. On a third metastasis needle biopsy sample, we performed transcriptomic analyses to identify potential molecular targets according to Lehman's molecular subclassification for triple negative breast carcinomas [58]. On the basis of molecular analyses for each patient, we tested drugs or drug combinations on the corresponding xenograft model. We were able to identify a potentially efficient chemotherapy regimen for each patient who progressed under 1–2 lines of chemotherapy. In each case,

the time to progression was longer than for previous lines of treatment [59, 60].

A similar strategy combining exome sequencing using next-generation-sequencing technology and individual xenograft models obtained from metastatic samples from 10 patients with different cancer types yielded a 77% rate of disease control [61].

Molecular biomarkers, integrated into complex pathways, are difficult to use directly in daily clinical practice. Individual xenografts from metastatic samples are an additional tool for in situ assessment of treatment efficacy.

In conclusion, patient-derived xenografts are preclinical models rediscovered as innovative pharmacological tools, both for the preclinical development of anticancer drugs and as individual models for personalized treatment of metastatic disease.

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Disclosure Statement

The authors do not have any conflicts of interest.

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