Preclinical and 'near-patient' models for the evaluation of experimental therapy in prostate and bladder cancer

Merbel, A.F. van de

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General Discussion and Future Perspectives

Every year, more than 2.4 million people are diagnosed with a urological cancer, including prostate, bladder cancer and kidney cancer. Despite extensive research in the uro-oncological field, the development of therapy resistance and the formation of distant metastases represent major clinical challenges for the adequate treatment of prostate and bladder cancer patients. Improved preclinical model systems that allow the identification of new therapeutic modalities are urgently needed to address these clinical challenges and provide better mechanistic understanding of tumor progression, metastasis and therapy resistance.

In this thesis, different preclinical strategies were explored aiming at the identification of putative novel therapies for prostate and bladder cancer. The first part of this thesis (**Chapter 2** and **Chapter 3**) describes the generation of preclinical, patient-derived model systems of prostate and bladder cancer. In **Chapter 2**, an overview is provided of the most commonly used patient-derived model systems for urological tumors, and a framework on how these patient-derived tumor models can be employed to address preclinical and clinical unmet needs is presented. In **Chapter 3**, we developed and optimized the culture of *ex vivo* tumor tissue slices and employed this model to detect anti-tumor responses of chemotherapeutic agents Docetaxel and Gemicitabin. Subsequently in **Chapters 4**, **5** and **6**, we describe the use of multiple preclinical translational models, including patient-derived tumor models. In **Chapter 4** and **5** the translational potential of the approved antipsychotic drug penfluridol was determined in bladder and prostate cancer. In **Chapter 6**, the use of oncolytic reovirus *jin-3* as putative novel therapeutic strategy for the treatment of prostate is investigated. Finally, in **Chapter 7**, we describe a novel preclinical screening strategy based on E-cadherin (re)induction and inhibition of invasion for the identification of a new class of small molecules for the treatment of aggressive epithelial cancers.

Patient-derived tumor models as a tool to identify novel therapies for urological tumors

Despite extensive preclinical research, the impact of preclinical research in clinical (uro)oncological practice has remained limited over the years. It has been estimated that only 0.0001% of all compounds investigated during preclinical research eventually reaches the clinic (1). Moreover, less than 5% of all anti-cancer compounds in phase I studies becomes approved by the FDA/EMA, and even less drugs are being implemented into clinical practice (i.e. the valley of death) (1). Multiple reasons for these high drug attrition in clinical studies have been reported in literature including a reduced drug efficacy in clinical studies, toxicology and clinical safety issues, commercial reasons, adverse pharmacokinetics and bioavailability profiles and formulation problems (2). These high drug attrition rates highlight the need for improved preclinical disease models for the identification of anti-drug candidates (3).

The development of clinically relevant preclinical models represents a major challenge for urological cancers, especially for prostate cancer. This is reflected by the relatively low number of existing preclinical models and the limited success rate of establishing novel preclinical tumor models, despite the high prevalence of these cancers (4). In this thesis, we have developed multiple near-patient models and exploited these models for the identification of novel therapies for prostate and bladder cancer. In contrast to 'traditional' preclinical tumor models, patient-derived tumor models use freshly isolated patient biopsies in order to better capture inter- and intrapatient heterogeneity and crucial tumor-stroma interactions, including the interaction with the immune system. Furthermore, prostate and bladder tumors are often multifocal, meaning the presence of multiple tumor foci with a different aggressiveness (5-7). In order to fully capture the multifocal nature of prostate and bladder tumors, it is of crucial importance to use patient biopsies from multiple sites when generating patient-derived tumor models.

Each preclinical model, including near-patient models, has its own inherent advantages and disadvantages and has the potential to address a specific combination of (pre)clinical unmet needs (**Chapter 2**). In order to address the (pre)clinical unmet needs, we used multiple patient-derived model systems models in parallel when testing the efficacy of novel experimental compounds (**chapter 4, 5** and **6**).

Despite the limited number of clinical biopsies, we observed heterogenous anti-tumor responses upon treatment with penfluridol and oncolytic reovirus in **Chapter 4, 5** and **6**. These results are in line with multiple studies reporting heterogenous drug responses in patient-derived models including PDX models, three-dimensional cultures and *ex vivo* cultured tumor tissue slices (8-11). This heterogeneity in patient-derived tumor models reflect the clinical reality in urological cancer patients where drug responses are notoriously heterogeneous (12).

The studies presented in this thesis employed patient-derived tumor models in a preclinical setting for drug development and screening purposes. However, employing patient-derived tumor models in a clinical setting could fully unleash the potential of these models. Traditional personalized medicine relies on performing genomic analyses in order to reveal druggable targetable driver mutations in cancer (13). However, focusing on genetic alterations alone may not be sufficient to predict personalized therapy responses since non-genetic mechanisms such as epigenetics and crucial tumor-stroma interactions are not captured by these genomic analyses (14). This notion was further reinforced by clinical studies where targeted therapy was based on the outcome of genomic analyses (15, 16). In these studies, only 38% of all patients had actionable mutations and only 18% had access to a relevant targeted therapy (16). Among these patients, only a small subset of patients displayed clinical benefit after treatment with the targeted therapy, with a clinical response rate of 2-38% (16). This suggests that targeted therapy based on genetic alterations results in limited clinical responses and other approaches should be considered.

In contrast to traditional precision medicine, functional precision medicine includes the strategy whereby tumor cells derived from fresh patient biopsies are directly exposed to drugs in order to obtain immediate, functional and translatable personalized information (17). To date, functional personalized medicine is currently not applied in clinical uro-oncological practice (17). In order to enable application in a clinical setting, clinical validation of near-patient preclinical models is of crucial importance. Other practical considerations need to be considered prior to clinical application, including optimizing tissue handling protocols, obtaining sufficient amounts of tumor biopsy material for preclinical testing, determining optimal drug doses and treatment duration in near-patient models. Moreover, the outcomes of preclinical testing need to be translated to corresponding clinical outcomes in a standardized manner. The personalized information obtained from functional precision testing could be used for guiding personalized therapy.

In the future, combining functional precision medicine with sequencing strategies could result in the prioritizing drugs based on their functional effect. In parallel, mechanistic explanations could be identified by performing genomic analyses that are responsible for this functional treatment effect (14).

Drug repositioning; antipsychotic drug penfluridol as a promising anti-neoplastic agent

Drug development is an expensive, time-consuming process. It has been estimated that it takes 12-15 years and costs over 1 billion dollar to develop a new drug from bench-to-bedside (18). In addition, a substantial number of drugs fails during the drug development process and will never reach the clinic. Drug repositioning includes the use of already approved compounds for other purposes. Since the safety and toxicity profiles of repurposed drugs are already known, drug repositioning might represent a promising strategy in order to overcome these hurdles (19). In contrast to developing novel drugs, repurposing existing drugs for a new indication costs approximately 300 million dollar, whereas the average time to repurpose a drug from bench-to-bedside will take around 6.5 years (20).

Chapter 4 and **5** of this thesis describe that repurposing of the antipsychotic drug penfluridol results in the induction of anti-tumor effects in both prostate and bladder cancer. These results are in line with multiple epidemiological studies revealing a reduced incidence of several types of cancer, including bladder and prostate cancer, in schizophrenic patients (21-24). Anti-tumor effects of penfluridol have been reported in multiple solid and hematologic malignancies, including breast cancer, lung cancer, pancreatic cancer, glioblastoma and leukemia (25-30). In **Chapter 4**, we described that penfluridol can be safely administered via intravesical instillation in mice. Moreover, intravesical administration of penfluridol reduced orthotopic bladder cancer growth and metastasis formation *in vivo.* Due to the barrier function of the bladder, higher drug doses can be administered via intravesical instillation, without inducing the psychological and neurological side-effects of penfluridol. Therefore, intravesical instillation seem to represent a promising method to administer penfluridol to bladder cancer patients, especially for patients with NMIBC that have a high risk at progression towards MIBC. When using penfluridol as a novel treatment for NMIBC via intravesical administration, clinical studies are warranted to determine the most optimal formulation and dose of penfluridol in patients.

Initially, a phase I/IIa study in intermediate-risk bladder cancer patients should be performed. In case of administering penfluridol in (metastatic) prostate patients, oral administration would be the preferred route of administration in order to target disseminated tumor cells. However, oral administration might result in (neurological) side effects in this fragile group of patients, that have undergone multiple treatments previously. Common side effects of penfluridol might include emotional numbing, movement disorders, headaches, dizziness, nausea and weight changes (31). Phase II and III studies should be executed to determine the efficacy of oral administration of penfluridol in prostate cancer patients.

In conclusion, future clinical studies are required to determine the most optimal dosing and route of administration of penfluridol in bladder and prostate cancer patients before enabling clinical application. After promising outcomes of clinical studies, approval by the EMA/FDA and patenting takes place. Despite the advantages of drug repositioning in terms of drug development costs and time, patenting of repurposed drugs for a new indication and enforcing this patent are often difficult (19). This could potentially result in a reduced profit for pharmaceutical companies and therefore in less interest by pharmaceutical companies in drug repositioning (19). In case of off-patent drugs including penfluridol, a method-of-use patent can be filed which describes a new use or application of a drug.

Oncolytic viruses and their therapeutic potential for human prostate cancer

Recently, the use of immunotherapy has emerged as a viable treatment strategy in the treatment of different types of cancer (32). However, immunotherapy have largely remained unsuccessful in the treatment of prostate cancer (33). Prostate cancer is known to be an immunological cold tumor due to its immune suppressive tumor microenvironment and its low tumor mutational burden (36). Oncolytic virotherapy could represent a promising strategy to overcome this immune suppressive barrier in prostate cancer. Oncolytic viruses are known for their dual mode of action; besides being able to infect and kill tumor cells, oncolytic viruses are capable of activating the immune system (34, 35).

In **Chapter 6** of this thesis, we have found direct oncolytic effects of spontaneous reovirus mutant *jin-3* in multiple preclinical prostate cancer models, including patient-derived tumor models. These results are in line with previous studies reporting on the anti-tumor effects of (wildtype) reovirus in other tumor types, including pancreatic tumors (37, 38). Furthermore, we observed that upon treatment with *jin-3* reovirus, prostate cancer cells secrete inflammatory cytokines and induce the expression of interferon-stimulated genes (**Chapter 6**). This suggests that *jin-3* reovirus could induce a proinflammatory phenotype in prostate cancer cells.

The results in this thesis are in line with previous studies reporting the immunomodulatory effects of wildtype reovirus via the induction of tumor associated antigens, dendritic cell maturation, proinflammatory cytokines, T cell activation and NK cell activation (39-42). Since the work described in **Chapter 6** investigated the aspects of *jin-3* infection in an immunodeficient setting, additional research in immunocompetent models such as humanized mouse models and co-cultures of 3D cultures with immune cells is required to further dissect the immunomodulatory aspects of *jin-3* reovirus in human prostate cancer. These experiments are currently ongoing in follow-up studies.

Multiple phase I studies have reported safe administration of wildtype reovirus in cancer patients (43, 44). To date, it remains unclear whether mutant reovirus *jin-3* could be safely administered in cancer patients. The administration of wildtype reovirus as a monotherapy has yielded only limited clinical benefit in cancer patients (45). Most clinical trials testing wildtype reovirus included patients with advanced, metastatic disease. As a result, the potential therapeutic effect of wildtype reovirus in cancer patients with organ-confined disease or patients that are at a high risk of progression were not included. Moreover, most trials focused on progression-free survival as outcome parameter and did not include the other outcome parameters during oncolytic virotherapy, e.g. the induction of innate and/or adaptive anti-tumor immune responses (45).

Since most of the clinical trials have reported only modest effects of wildtype reovirus on progression free survival, current clinical studies are focusing on combining wildtype reovirus with other treatment modalities. Interestingly, a preclinical study has revealed that wildtype reovirus has synergistic effects with docetaxel in prostate cancer cells *in vitro* (46). Moreover, treatment with wildtype reovirus sensitized prostate cancer cells to PD1-PDL1 inhibition (47).

These results indicate that combining reovirus with other treatment modalities could fully unleash the therapeutic potential of oncolytic reovirus and might represent a promising treatment strategy for prostate cancer. The effect of *jin-3* in combination with other treatment modalities remains unknown to date and future studies are required to investigate the effect of *jin-3* in combination with other treatment modalities. These studies have now been initiated in a follow-up project for multiple oncolytic viruses developed at LUMC.

Obviously clinical application of *jin-3* reovirus in (prostate) cancer patients requires multiple (bio)safety aspects need to be addressed first, including viral shedding to blood, stool and urine. A previous study did not reveal viral shedding of wildtype reovirus in blood, urine and stool samples upon intravenous administration when performing RT-PCR based on 25 cycles of amplification (43). However, when performing a more sensitive RT-PCR based on 35 cycles of amplification, low levels of reovirus could be detected in the blood, urine and saliva in a small number of patients (43). These results highlight the importance of investigating these biosafety aspects.

Small molecule anti-invasive compounds in aggressive epithelial tumors

In cancer, invasion of local tissue and the formation of distant metastases represents a major clinical problem and largely determines a patient's clinical outcome. Epithelial-to-mesenchymal transition (EMT) represent one of the biological processes through which epithelial cancer cells acquire invasive and aggressive characteristics (48-50). EMT is a complex, transient and reversible biological process. As a result, partial EMT, a hybrid state that is characterized by simultaneous expression of both epithelial and mesenchymal markers, has been reported in multiple cancer types (52). EMT is associated increased stemness, therapy resistance, angiogenesis, immune suppression and a poor prognosis in multiple types of cancer (48, 49). Therefore, targeting of EMT represents an interesting anti-cancer strategy. One of the hallmarks of EMT includes downregulation of epithelial marker E-cadherin (50). In **Chapter 7** of this thesis, we screened multiple compounds for their ability to (re)induce E-cadherin expression and to inhibit tumor cell migration. The PROAM02-class, including compound PROAM02-0008, was identified to induce E-cadherin expression and to reduce invasiveness and metastases formation *in vivo.*

At this moment, multiple EMT-inhibitors are investigated in clinical studies (51). Due to the complex and transient nature of EMT, timing and duration of EMT-targeting therapies plays a crucial role (53). Moreover, since EMT is involved in several physiological processes such as wound healing and tissue remodeling, careful monitoring of the side effects is of crucial importance when administring EMT inhibitors in cancer patients (54). Current debates are ongoing which subgroup of patients will benefit the most from anti-EMT therapy. Administration of EMT inhibitors in patients suffering from organ-confined disease could prevent metastatic spread to distant sites. In this way, EMT-inhibitors could aid in successfully surgically resecting the tumor (51).

At later disease stages, EMT inhibitors could reduce the formation of circulating tumor cells, prevent colonization of distant sites by circulating tumor cells and could reduce metastasis-to-metastasis seeding. Furthermore, administration of EMT inhibitors at a later stage could result in an increased sensitivity to chemotherapy and immune therapy (51). EMT inhibitors could promote MET, which has been associated with the outgrowth of tumor cells at distant sites. Therefore, EMT-inhibition could potentially result induce proliferation and the faster outgrowth of tumor cells at distant sites. It is of crucial importance to closely monitor the tumor burden of the patients during EMT-inhibition therapy. Since EMT plays multiple roles in different stages of cancer progression and other physiological processes, EMT inhibitors should be administered in a clear therapeutic window. Therefore, applying a personalized and stage-specific approach when prescribing EMT-inhibitors would be recommended.

During administration of compounds belonging to the PROAM02 class *in vivo*, compound precipitation was often observed. It has been reported that over 75% of all drug candidates for drug development have a poor solubility profile (55). A poor solubility profile could result in reduced absorption, decreased bioavailability and increased gastrointestinal toxicity, resulting in a narrow therapeutic index (55). Improving water solubility is crucial for clinical administration. Improving water solubility of the PROAM02-0008 compound with different solubilization strategies seems, therefore, a prerequisite for successful clinical translation (56).

Future recommendations

In this thesis, we have employed several preclinical strategies to identify antipsychotic drug penfluridol, mutant oncolytic reovirus *jin*-3 and small molecule PROAM02-0008 as novel drug candidates for prostate and/or bladder cancer. In this thesis, the use of patient-derived tumor models alone or in combination with traditional preclinical tumor models, has proven to be valuable tool in preclinical prostate and bladder cancer research. In future preclinical drug development, it would be beneficial to employ multiple patient-derived tumor models in parallel, instead of relying on traditional preclinical models alone. The refinement of current existing near-patient models, for instance by co-culturing of three-dimensional cultured tumor cells with stromal cells and/or immune cells, the development of humanized mouse models and automated analysis platforms based on spatial and molecular profiling, represent a promising developments.

At this moment, it is not entirely clear whether routinely implementing near-patient models in preclinical drug development will result in a more efficient drug development process. Future studies, including performing co-clinical trials, are required to elucidate whether the use near-patient models will result in lower drug attrition rates. Besides the use of near-patient models, other preclinical strategies (e.g. screening for E-cadherin (re)induction and inhibition of migration) and the use of existing drugs for novel indications (i.e. drug repositioning) represent promising preclinical approaches that could potentially lead to higher success rates and a faster drug development process for urological cancers.

To date, personalized medicine and patient-derived tumor models are not routinely implemented in the uro-oncological clinical practice. Clinical application of patient-derived tumor models could aid in clinical decision making for urological cancers. Prior to routinely implementation into clinical practice, the execution of co-clinical trials is essential to validate the preclinical outcomes. After clinical validation, clinical application of near-patient models alone (i.e. functional precision medicine) or combined with genomic analyses could result in better medication effectiveness, a reduction in the adverse events and an improved disease management in patients suffering from urological cancers. In order to enable (functional) personalized medicine for prostate and bladder cancer patients in the future, it is of pivotal importance to establish interdisciplinary collaborations between scientists, urologists and pathologists to enable free exchange of tumor material, clinical data and preclinical knowledge.

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