Novel tools and applications in tumor models

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Various tumor models for the evaluation of novel cancer therapeutics

- Traditional xenograft models
  - Subcutaneous xenograft models
    - Implantation of human tumor cells subcutaneously into immunodeficient mouse
    - Relatively cheap, fast and easy to perform
    - Lack the correct tumor microenvironment
  - Orthotopic xenograft models
    - Implantation of human tumor cells into the organ of origin of immunodeficient mouse
    - Expensive, time consuming and technically challenging
    - Relevant tumor microenvironment, clinically relevant
    - May also include formation of metastases depending on cells used
- Syngeneic models (subcutaneous or orthotopic)
  - Implantation of mouse tumor cells into (immunocompetent) mouse
  - Tumors originating from their own species
  - Enable to study how immunotherapies act in a functional immune system
  - Similar technical advantages and limitations as with xenograft models
Various tumor models for the evaluation of novel cancer therapeutics

- **Patient Derived Xenograft Models**
  - Tissue from a patient’s primary tumor implanted directly into an immunodeficient mouse
  - Possess natural tissue architecture and composition
  - Expensive, time consuming and technically challenging
  - Often performed as subcutaneous models

- **Genetically engineered mouse (GEM) models**
  - Generated through the introduction of genetic mutations associated with particular human malignancies or transformation
  - Natural tumor microenvironment and immune competence
  - Tumors often mesenchymal instead of epithelial origin
  - May also include spontaneous carcinogenesis
Common features of various tumor models

- Study setups, parameters and endpoints
  - Typical duration between few weeks to few months
  - Immunodeficient or immunocompetent mice
  - Prevention, treatment or survival studies
  - Dosing of test compounds in efficacy studies from single dosing up to several times dosing per day
  - Dosing routes typically po, ip or sc depending on the formulation of test compounds
  - Anesthesia needed typically at implantation of tumor or cells, imaging and at sacrifice
  - Appropriate analgesia to minimize pain and distress, particularly at the end of in-life phase
Common features of various tumor models

- Various measurements for the assessment of disease progression and efficacy of therapy
  - During the in-life phase
    - Tumor burden by serial imaging (if labelled cells)
    - Body weight, cachexia, paraplegia
    - Soft tissues metastases by serial imaging (if labelled cells and soft tissue metastases occurred)
    - Osteolysis by radiography (if bone metastases occurred)
    - Biomarkers
  - At the end of the study
    - Histomorphometry (static and dynamic) of tumor and other tissue
    - Immunohistochemistry
    - Tumor volume and macroscopic findings
    - Molecular analysis
Common consequences of various tumor models

- Implantation of tumor cells or tumor tissue causes often severe disease
  - Cancer studies lead often cachexia, paralysis and pain at the end of the in-life phase
  - Vicious cycle in bone metastases studies lead severe osteolysis
- Earliest scientifically justified point for the termination of study is often when disease has severe symptoms
  - In efficacy studies traditional analyses such as histology and radiography requires visible changes at tissue level
  - In survival studies the type of the study itself
- Mode of treatment and study setup may cause further stress
  - Excessive handling and injection stress raises catecholamine levels thus effecting for example cardiovascular study outcomes such as blood pressure
  - Oral gavage dosing can induce bleeding in oesophagus
  - Fasting needed for biomarker measurements, usually at least 6 hours needed
How can we implement better 3R principles in tumor studies?

- Industry wants to cut costs, but at the same time have better outcomes and predictivity of the studies
- 95% of the anticancer compounds fail in clinical phases => Predictive value of all preclinical data is only 5%!
- Ethical concerns => growing criticism on painful animal experimentation
- Can we improve the predictivity of tumor models and concomitantly implement better 3R principles?
- Two case examples of novel tools and applications on how both of these issues can be implemented in tumor models:
Example 1: Using “early detectors” of disease progression

- Treatment of established bone metastases, MDA-MB-231 breast cancer xenograft study
- Groups (Final n= 14-16 per group)
  - Control, vehicle
  - Reference compound 1: Doxorubicin 2.5 mg/kg weekly i.p.
  - Reference compound 2: Zoledronate 100µg/kg once s.c.
  - Combination group: Sequential treatment (dox + after 24h zol)

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**Sacrifice;**
- Blood sample
- Radiography
- Non invasive-imaging → **Biomarkers**
- Blood sample
- Radiography
- GFP-imaging → **Biomarkers**
Example 1: Using “early detectors” of disease progression, biomarkers

Conclusions:

- Zoledronate prevents the increase of ostelolytic area
- TRACP 5b biomarker may predict tissue level (ostelolytic lesions) changes several days earlier before changes are visible
Example 1: Using “early detectors” of disease progression, imaging

Conclusions:
- GFP-labelled MDA-MB-231 breast cancer cells are visible throughout the animal
- GFP-imaging reveals both, bone and visceral metastases
- GFP-imaging may predict tissue level (ostelolytic lesions) changes few days earlier before changes are visible
Example 2: Using novel methods for long term dosage of animals, MedRod™

- MedRod™, Polymer based long term drug delivery systems
  - Typically 3 mm diameter, 10 mm long cylinder shaped rod
  - Size is dependable on the used substance, study type and duration of the study
  - Elastic, safe and well tolerated
  - Can be used for stable release subcutaneously (s.c) or even in vitro
  - Optimal release capacity for steroid substances such as dihydrotestosterone (DHT), testosterone and estrogen
  - Enables decreasing animal number to be used in the studies thus follows 3R principle

Fig 1: A) Cross-sectional magnified image of a 1 cm long 3 mm OD membrane covered MedRod™ filled with test compound; B) Elastic properties of a polymer rod; C) Magnified image of a test compound filled MedRod™ after 8 weeks experimentation.
MedRod™ drug delivery systems

**Benefits of MedRod™**

- Adjustable sustained drug release from weeks to several months
- Utilities biocompatible, biostable soft matrix that remains unchanged in tissue
- Soft nature of MedRod™ minimizes irritation and maximizes comfort
- Small in size and thus easy to assemble
- Removable at any time of study, if sudden termination of drug delivery desired
- No replacement required during the study compared to many other substance releasing devices
- Save time and money by eliminating the need for frequent animal handling
MedRod™ drug delivery systems

Possible indications
- Release of hormones in hormone dependent cancer and metabolic disease animal studies
- Release of analgesia in preclinical animal studies
- Release of growth hormone in nerve injuries animals studies
- Release of antifibrotic medication and steroids in heart failure animals studies

The effect of DHT on orthotopic LNCaP tumor growth at 8 weeks, conventional dosing
A pilot study: The effects of DHT MedRod™ in three different mouse strain

- 8 weeks study, n = 7 in each group
- Serum DHT measurements by kit chemistry at various time points
- Liver and prostate weight at sacrifice
- Body weights and macroscopic findings during the study
- Groups
  1) Harlan athymic wo treatment
  2) Harlan athymic with DHT MedRod™
  3) CRL NMRI wo treatment
  4) CRL NMRI with DHT MedRod™
  5) CRL foxnude wo treatment
  6) CRL foxnude with DHT MedRod™
Results of DHT MedRod™ study

Groups
1) Harlan athymic wo treatment
2) Harlan athymic with DHT MedRod™
3) CRL NMRI wo treatment
4) CRL NMRI with DHT MedRod™
5) CRL foxnude wo treatment
6) CRL foxnude with DHT MedRod™
Conclusions of DHT MedRod™ study

- During the 8 weeks study DHT was released continuously from polymer tubes as assessed by DHT measurements
- DHT release was stable with low variance
- The release of DHT was physiological and anabolic as assessed by increased prostate weights
- Liver weights were slightly elevated due to increased metabolic activity, but no pathological findings were observed
- MedRod™ systems were well tolerated during the in-life phase
- Animals had no signs of inflammation upon sacrifice
- Based on preliminary results, MedRod™ drug delivery systems can improve predictability and accuracy of preclinical models providing concomitantly option to decrease the use of research animals
Summary

- Animal studies are required before human administration of new anticancer treatment.
- Various animal models of cancer are irreplaceable link between in vitro studies and clinical data.
- Lack of valid preclinical efficacy models and tools is particularly high in the field of oncology as 95% of the potential anticancer drugs fail in clinical phases.
- Efforts to develop more predictive in vivo models and concomitantly implement better 3R principles are often controversial.
- Key stakeholder is industry and its efforts to increase predictivity and cut costs.
- Novel applications in the field of noninvasive imaging and biomarkers as well as novel tools such as MedRod™ may have the potential to increase predictivity and concomitantly implement better 3R principles in tumor in vivo studies.