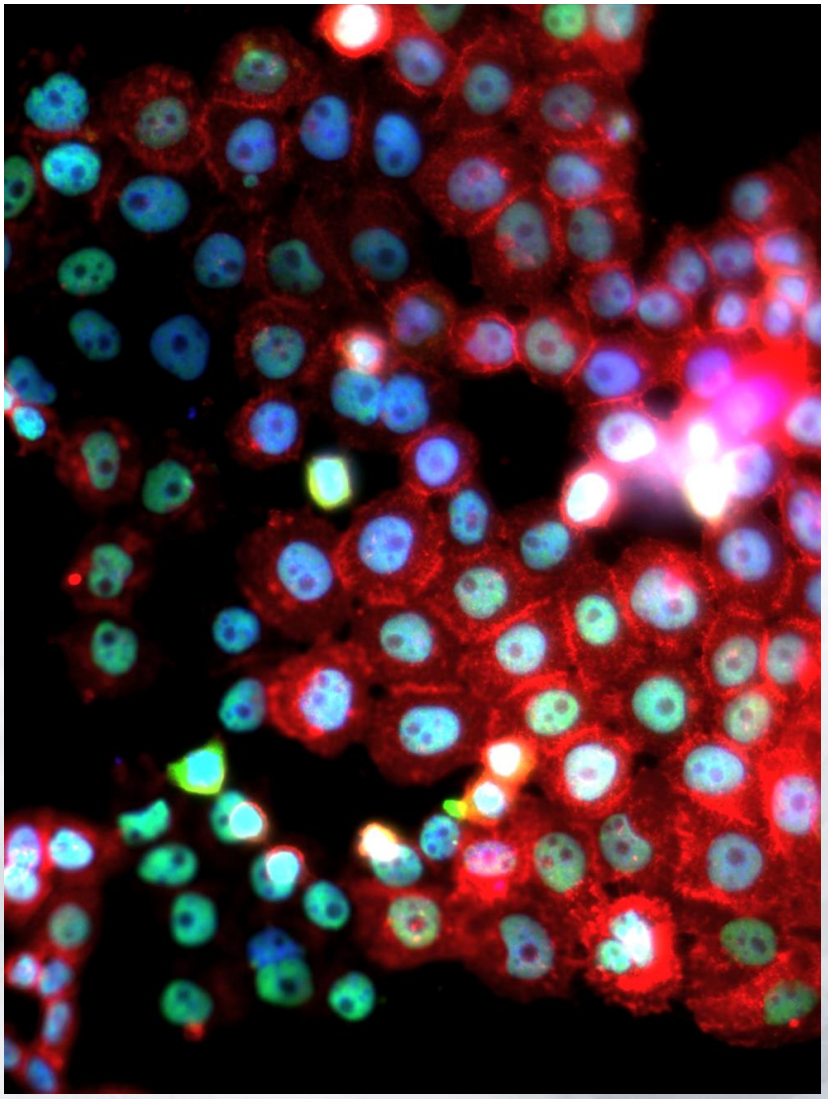
Patient derived models for breast, colon and prostate cancer

Advantages and limitations of stable, commercially available cell lines



Commercially available (stable) cancer cell lines

Advantages:

- Genetically stable
- Well-defined (genetics, transcriptomics and proteomics)
- Useful for mechanistic and molecular studies

However…

A Disease: Model vs Reality

Major differences between clinical cases and stable cell line models

COMMERCIALLY AVAILABLE CELL LINES	VS	CLINICAL CASES
Lost	Amplification	Present
Lost	Specific Mutations, e.g heterozygous Tp53	Present
Lost	Cancer stem cells	Present
Restricted	Genetic background	Diverse
Mostly Homogenous	Cellular composition	Heterogenous
Not applicable	Invasiveness	Required for tumor growth
Defined medium/matrix	Environmental regulation	Complex
Not applicable	Metastasis	Multiple sites
Rapid	Growth rate	Predominantly slow
Instantenous	Treatment duration	Months

More precise tumor models are needed to ensure
better scientific results and higher predictability of clinical trials!

Requirement for primary cell line models in the literature

„For decades, immortal cancer cell lines have constituted an accessible, easily usable set of biological models to investigate cancer biology and explore the potential efficacy of anticancer drugs. However, numerous studies suggest that these cell lines poorly represent the diversity, heterogeneity and drug-resistant tumors occurring in patient. The derivation and short-term culture of primary cells from solid tumors have thus gained significant importance in personalized cancer therapy.” (A. Mitra, *et. al.* **2013**, Trends Biotechnol.,31(6): 347-354)

„Although cultured cells can be used to study many aspects of cancer biology and response of cells to drugs, this study emphasizes the necessity for new in vitro cancer models and the use of primary tumor models in which gene expression can be manipulated and small molecules tested in a setting that more closely mimics the in vivo cancer microenvironment so as to avoid radical changes in gene expression profiles brought on by extended periods of cell culture.” (JP Gillet *et. al*. **2011**, PNAS, vol. 108, no. 46)

„The few bona fide cell lines, almost all derived from metastases, do not span the range of prostate cancer phenotypes, and in particular are not representative of primary adenocarcinomas of the prostate. Furthermore, the question of how extensively long-term culture alters the biological properties of cell lines is always lurking. For these and other reasons, primary cultures of malignant prostatic cells and their normal epithelial counterparts are sought.” (DM Peehl *et al*. **2005**, Endocrine-Related Cancer, ***12,*** p.19-47)

New tools to support your drug discovery

More precise cancer models

- **Primary cancer cell lines** click for more details

Cells isolated from tumor slices derived directly from patient, cultured *in vitro* for only the first couple of passages to avoid cellular adaptation

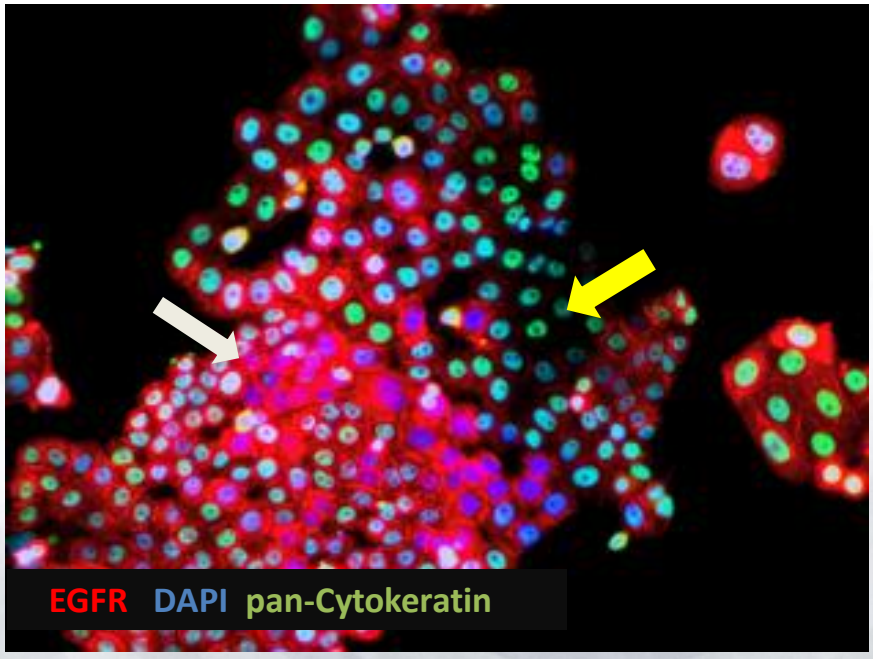
- **Human cancer xenografts (mice)** click for more details

Cancer samples are injected directly into the immunocompromised mice

- **Genetically Engineered Mouse Models (GEMMs)** click for more details

Mice carrying the mutations contributing to cancer development

No adaptation→
Primary human cancer cell lines

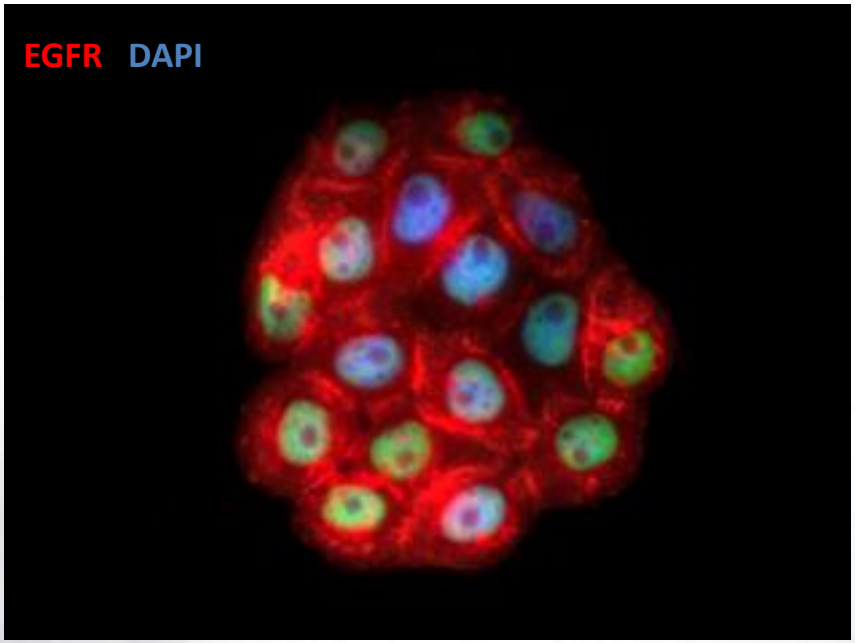


Immunocytochemical analysis of human primary breast cancer cells in vitro (CLTH/BC, Cat no. CL 04002-CLTH). White arrow indicates cells with excess EGFR, in contrast to cells indicated by yellow arrow.

- During stabilization of the commercially available cell lines, cells are artificially immortalized and adapt to an *in vitro* environment (oxygen and metabolite supply changes and appearance of additional mutations)
- In effect, amplification of genes is commonly lost during cell line stabilization when compared to tumors and primary cancer cell lines
- Primary human cancer cell lines do not have sufficient amount of time to undergo adaptation to an *in vitro* environment, therefore they more closely reflect *in vivo* state

Heterozygosity →
Primary cancer cell lines

- The percentage of surgical and biopsy specimens described as single heterozygous mutation of TP53 is very high (35%) compared to only 10% in stable cell lines
- Commercially available cell lines show a lower proportion of wild-type TP53 retention because of more frequent 17p LOH and secondary heterozygous TP53 mutations
- Primary cancer cell lines remain heterozygous for several passages, offering a good model for studies



Exemplary immunocytochemical analyses of human primary breast cancer cells (CLTH/BC, Cat no. CL 04002-CLTH) *in vitro* – TP53 nuclear accumulation (green signal)

Heterogeneity →
Primary cancer cell lines

- Composition of tumors is diverse, with differences between individual cells of the same origin (e.i. protein expression levels)
- During stabilization process only the fittest cells survive, making the stable cell lines more uniform
- Short period of *in vitro* culture is insufficient for selection process to take place in primary cancer cell lines

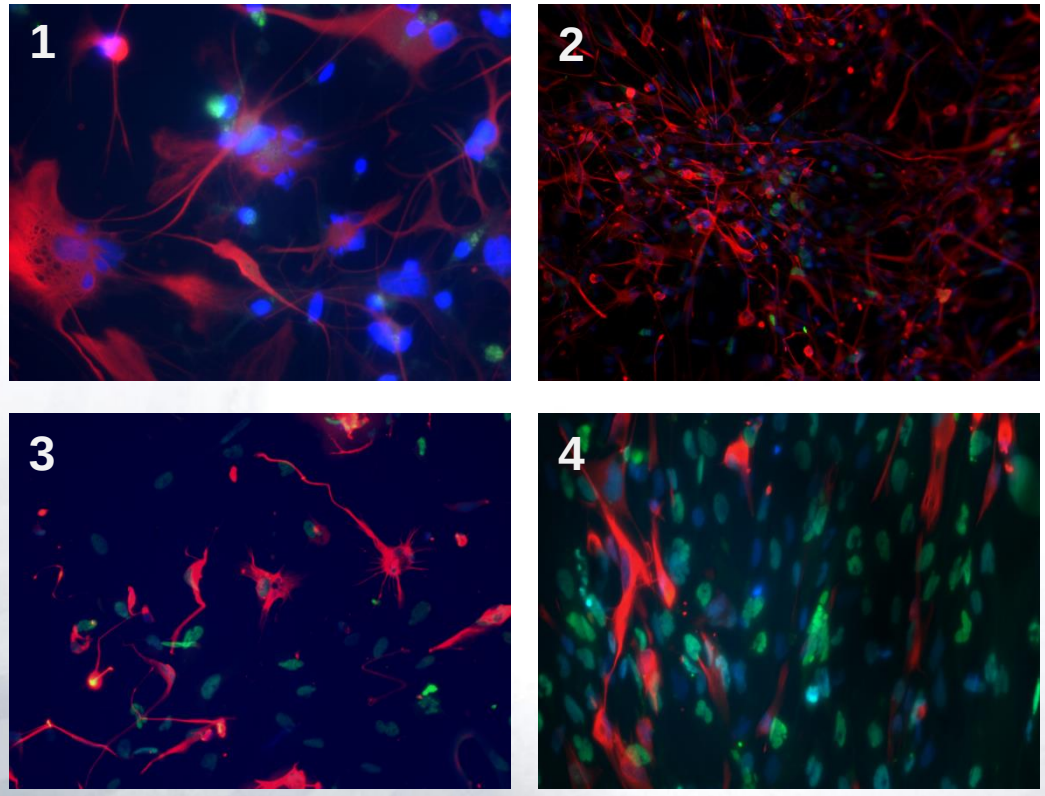
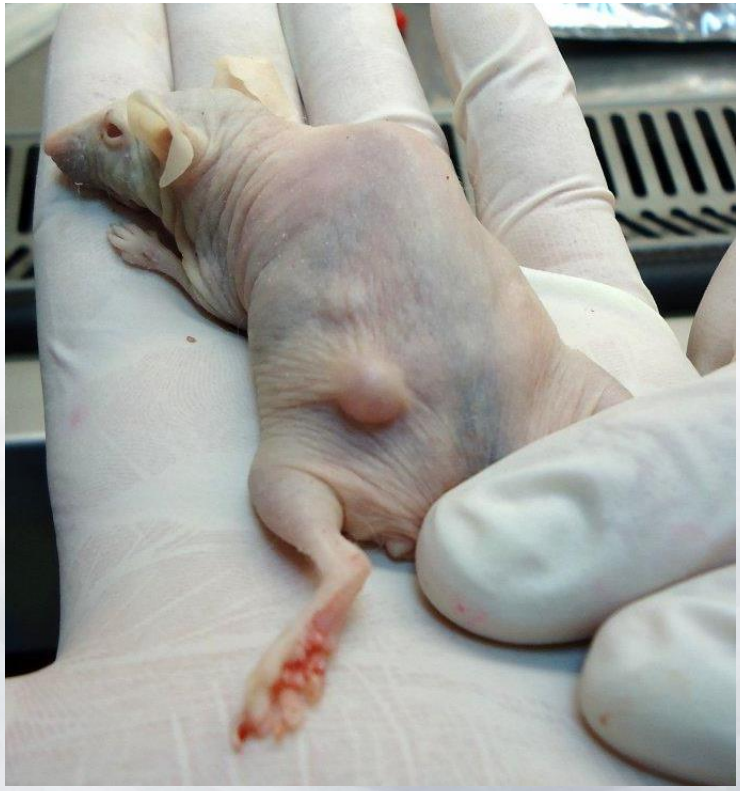


Fig. 1-4 show neoplastic cells selected from various fragments of tumor

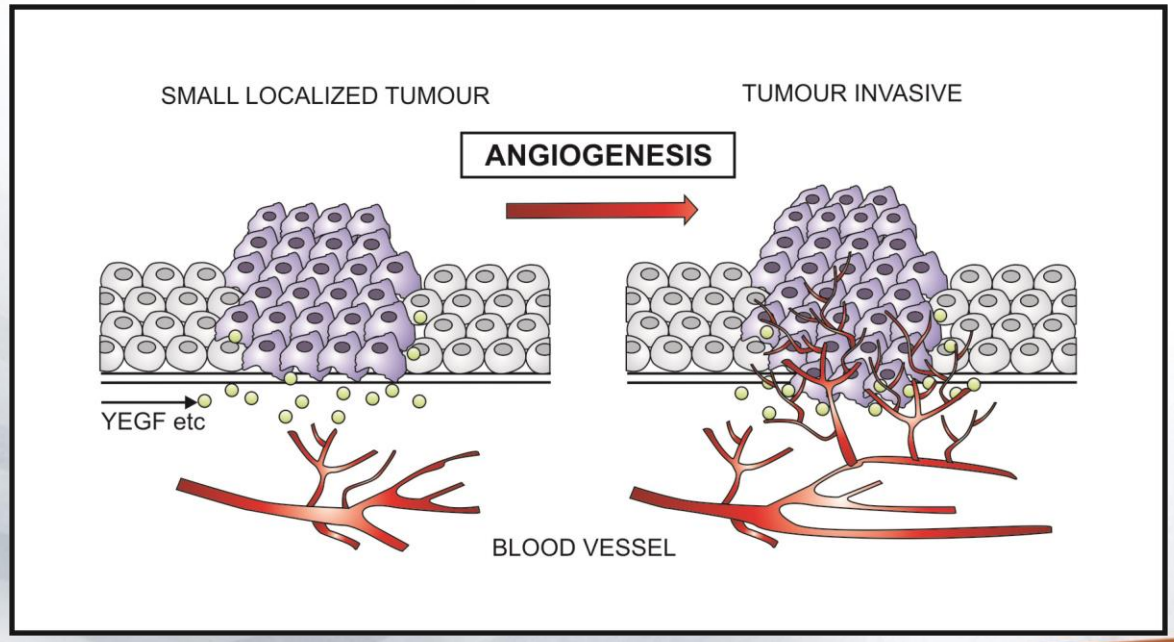
Invasiveness → Human xenografts



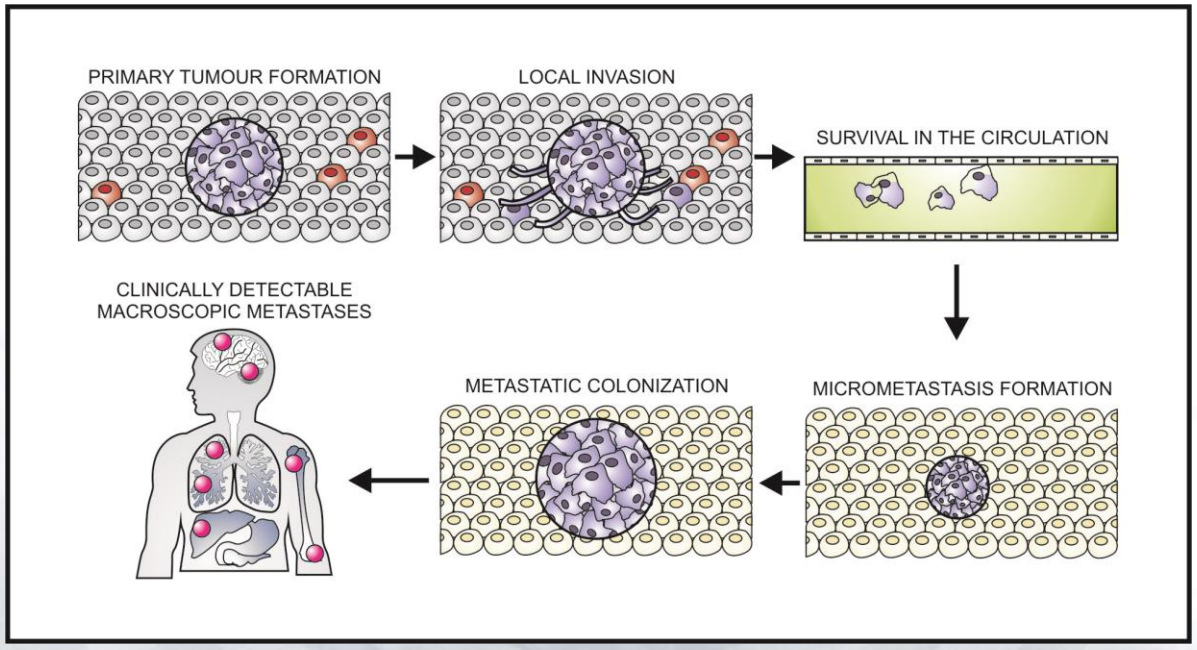
- In order to grow, tumor cells need to expand, invading the surrounding tissues
- Stable cell lines grown in a monolayer or suspension, in contrast to xenografts, do not reflect the complexity of the 3D environment
- Tumors xenografted into mice face complex environment, mimicking the invasion process in human body

Environmental regulation → Human tumor xenografts

- Growing tumors have to cope with pressure from surrounding tissue and their secretions, whilst securing increased oxygen and metabolite supply
- It is nearly impossible to mimic the environment of the tumor under *in vitro* conditions
- Xenografts reflect the environment of the tumor, providing more precise model to examine *in vivo* therapeutic responses to drugs

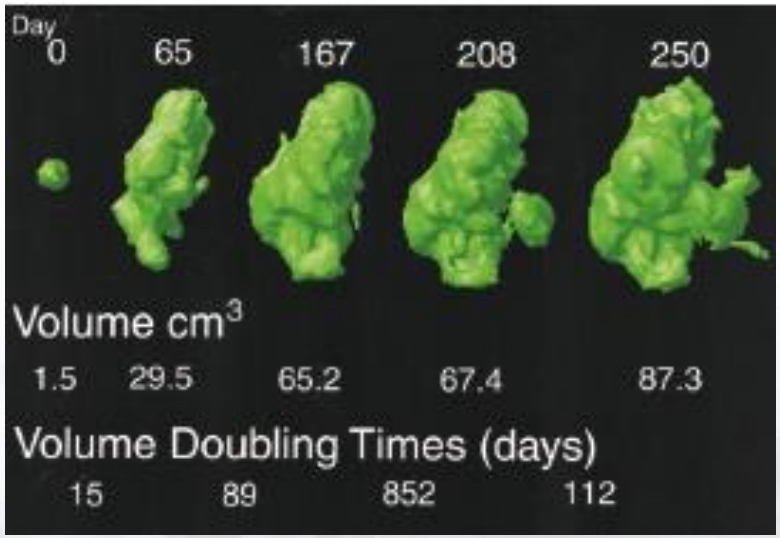


Metastasis→GEMMs



- Numerous cancers are lethal due to their metastatic nature and secondary tumor mortality
- Study of the process of metastasis requires orthotopic tissues as well as means of mobility (e.i. bloodstream)

Growth rate→ GEMMs



Tracking Tumor Growth Rates in Patients with Malignant Gliomas: A Test of Two Algorithms, AJNR Am J Neuroradiol 22:73–82, January 2001

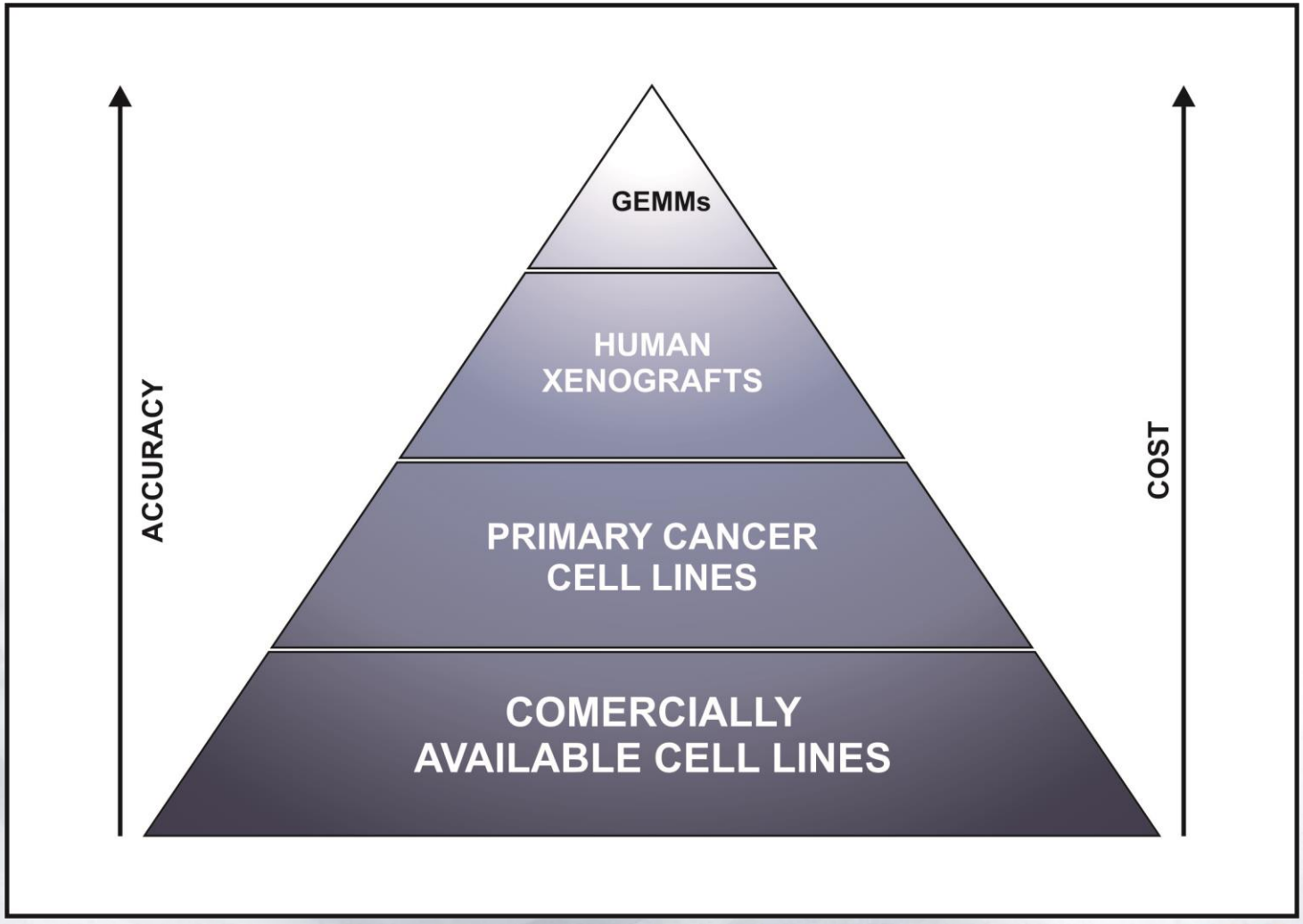
- Growth of tumors is impaired by the immune system, lack of vascularization and spatial restrictions
- While tumor cells generally proliferate faster compared to normal body cells, they do not come even close to the proliferation rate of established cell lines
- GEMMs model is a tool which retains poliferation rate observed *in vivo* (in patient)

Treatment duration → GEMMs

- Numerous drug candidates fail due to excessive cytotoxicity or inability to reach tumor cells

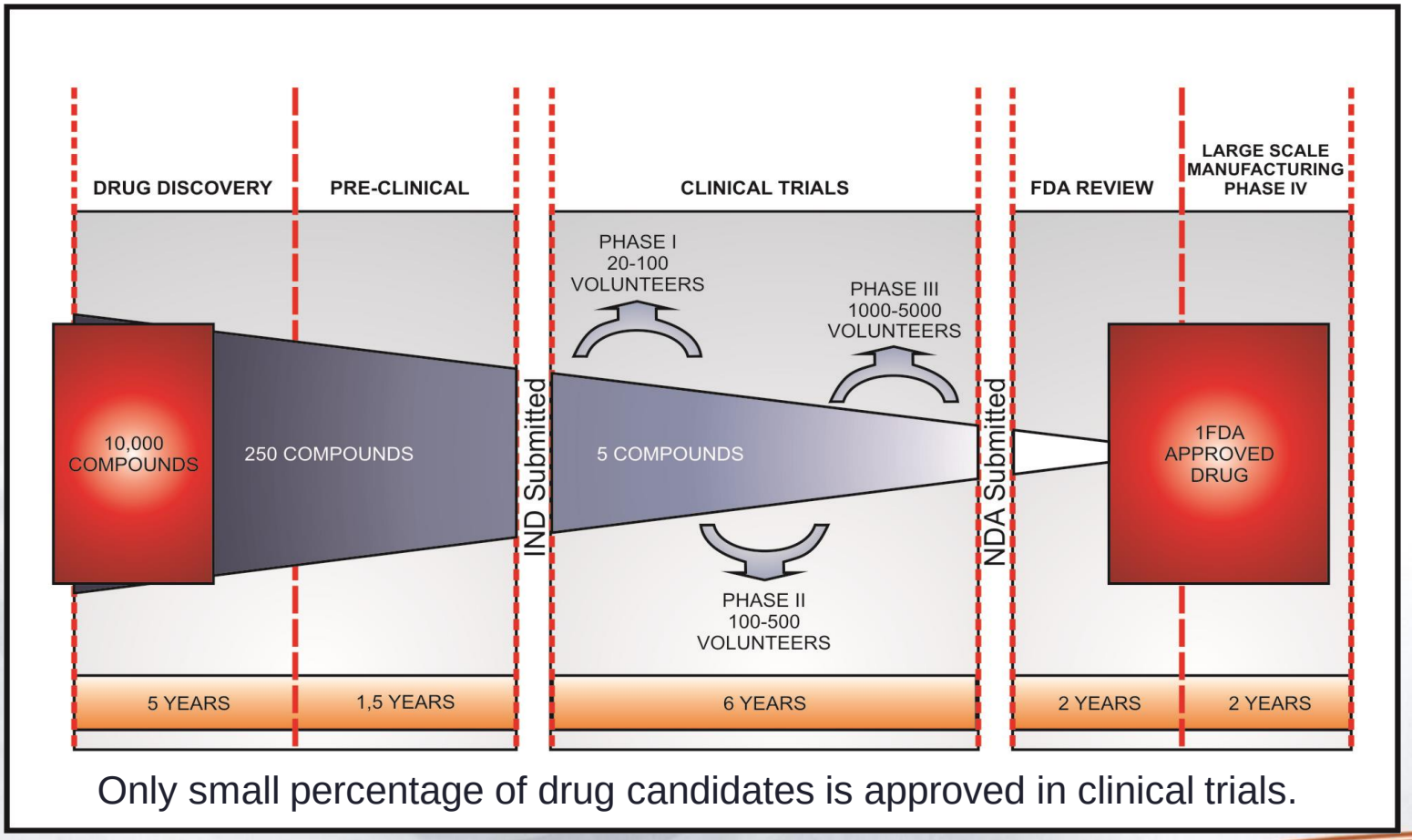
- Only models as complex as human body (e.g. GEMMs) can depict the difficulty of delivering the right concentration of the drug to the correct site in the body

Model accuracy vs cost



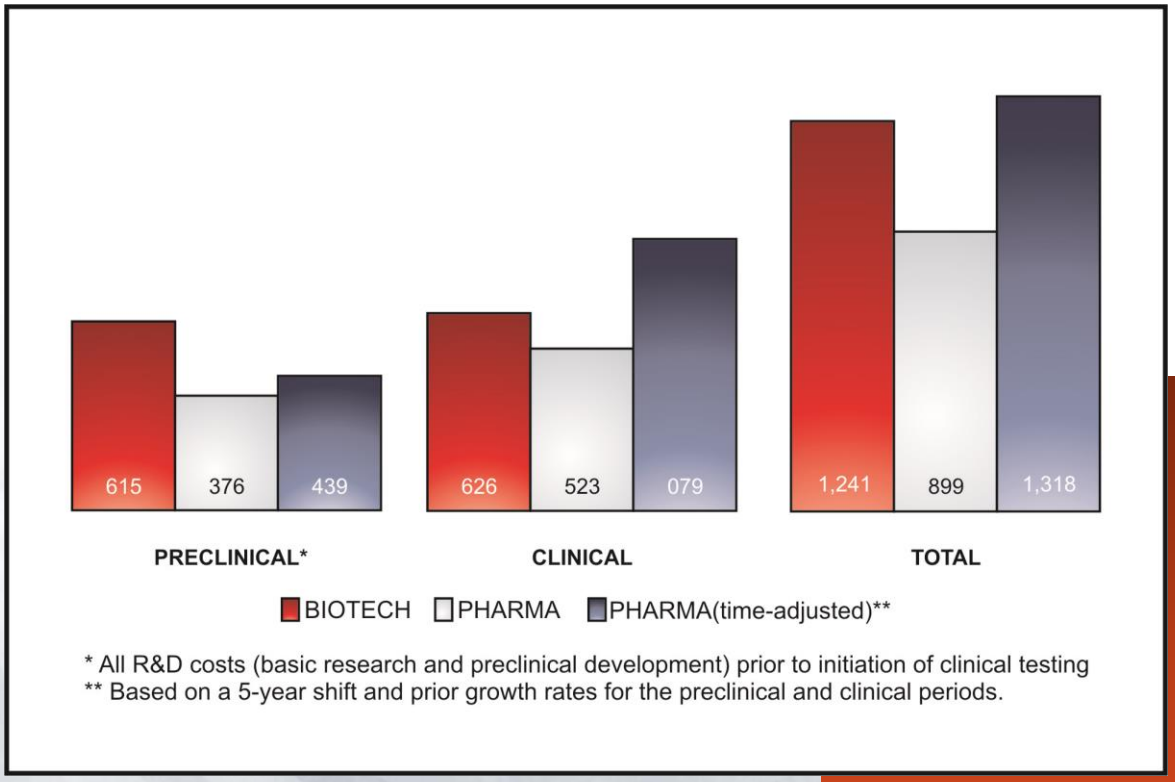
New Cancer Models
- why needed

Biopharmaceutical drug development: Attrition



Only small percentage of drug candidates is approved in clinical trials.

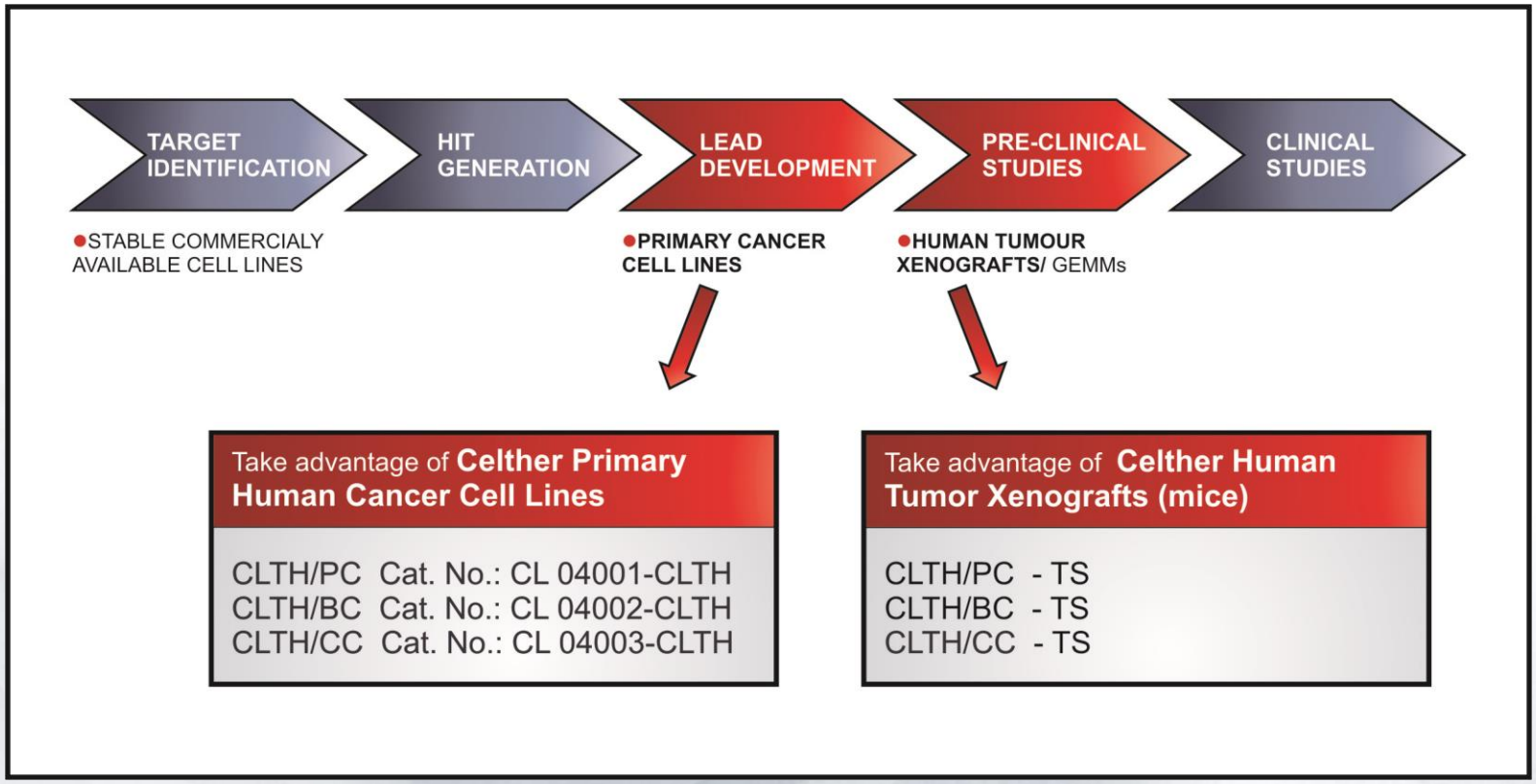
Capitalized cost estimates per new molecule



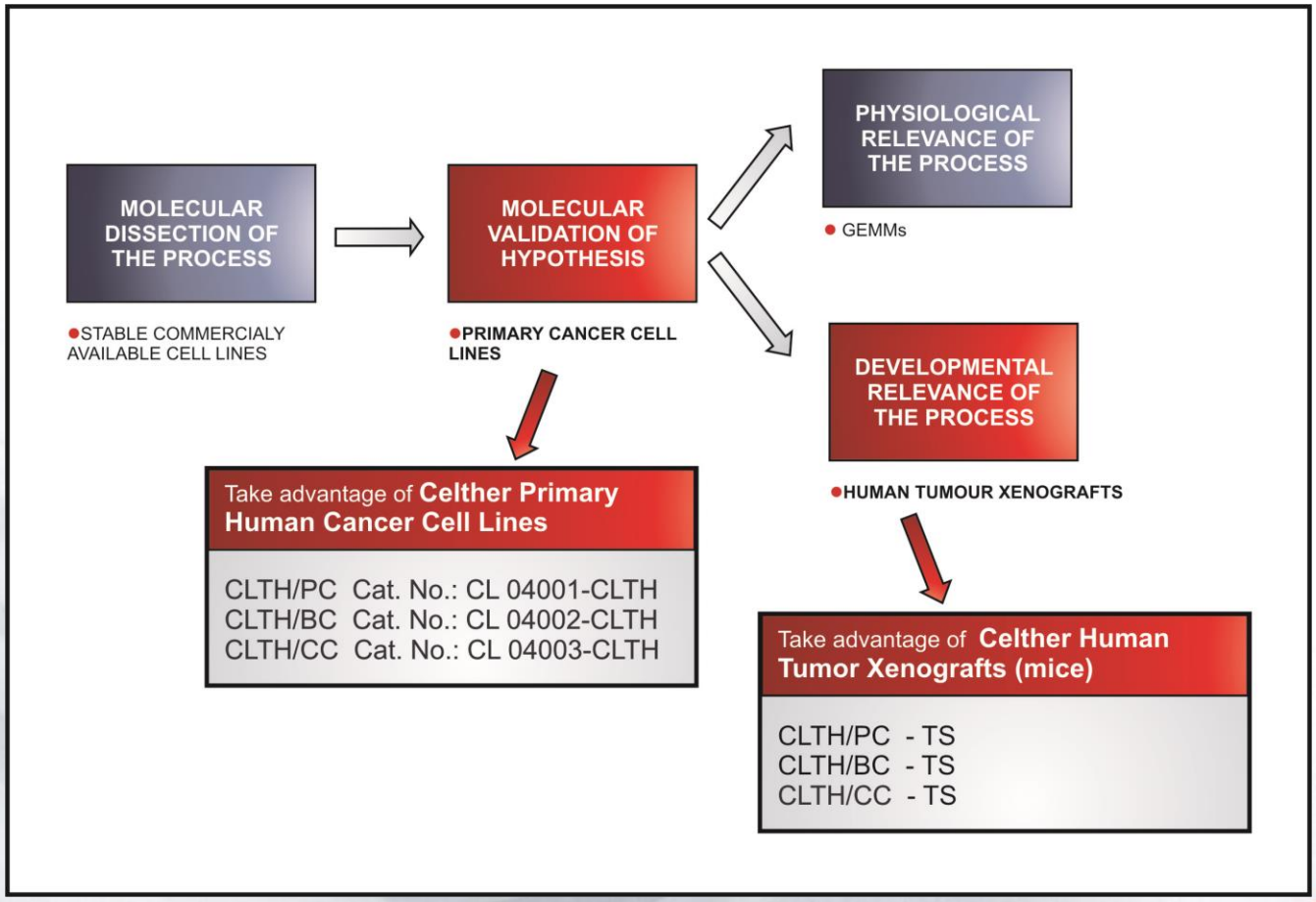
Most drug candidates fail costly clinical studies. Patient derived models are an effective tool at avoiding generation of additional unnecessary costs.

Exemplary use of cancer models

Exemplary use of models in high-throughput-screening pipeline



Exemplary use of models in primary research project pipeline



Celther POLSKA

Primary cancer cell lines are more clinically relevant model

FEATURE	PATIENT (CLINICAL CASES)	HUMAN PRIMARY CANCER CELL LINES	COMMERCIALLY AVAILABLE STABLE CELL LINES
Amplification	Present	Present	Lost
Cancer stem cells	Present	Present	Lost
Specific Mutations, e.g, EGFRvIII	Present	Present	Lost
Genetic background	Diverse	Diverse	Restricted
Cellular composition	Heterogenous	Heterogenous	Mostly homogenous
Invasiveness	Required for tumor growth	Reflects original tumor	Not applicable
Environmental regulation	Restricted	Diverse	Not applicable
Growth rate	Predominantly slow	Rather slow	Rapid
Oncogene induced apoptosis /senescence	Sensitive	Sensitive	Resistant
Biology	Divergent	Like *in vivo*	Marginal representation (anomalies)

Primary cancer cell lines are more clinically relevant model

Only 10% of tumors are able to give rise to stable cell lines, less than 0.1% in case of prostate cancers.

Taking into consideration that milions of clinical cases of heterogenous tumors have been reported and only few stable cell lines (e.g. prostate cancer cell lines) were established, we can describe those lines as anomalies.

This means, that they do not reflect situation in clinic and can not be used to examine therapeutic responses to drugs or even general research.

Primary cancer cell lines

Application:

- Assessment of the effectiveness of targeted therapy on cultured cells prior to *in vivo* validation
- Best tool to confirm your preliminary observations made in stable, commercially available cell lines
- Suitable for basic research on tumor biology

Advantages:

- The biological response may be closer to an *in vivo* situation than the one obtained with cell lines
- Physiologically relevant to test the predictions made using immortalized cell lines
- Usually retain numerous characteristics of the differentiated cells *in vivo*
- Heterozygosity
- Heterogeneity
- Fairly well reflect composition of tumors *in vivo*

Return

Human tumor xenografts (mice)

Application:

- Examination of *in vivo* therapeutic responses to drugs
- Increasing understanding of factors affecting tumor growth
- Depict complexity of genetic and epigenetic abnormalities that exist in the human tumor population
- Development of individualized molecular therapeutic approaches

Advantages:

- Fast growing tumors
- Results of response to therapy can be obtained after few weeks of therapy
- Multiple therapies can be tested from a single tumor biopsy
- Data from tissue microarrays and genetic microarrays can be readily obtained from the human biopsy and xenograft tissue, before and after drug therapy, for extensive analysis before the patient is subjected to therapy that may not work
- Orthotopic xenografts can be appropriately placed to reproduce the organ environment in which the tumor grows, so that the effect of the tumor on its microenvironment can be modulated (with the exception of certain T-cell populations)
- Stroma from the human tumor microenvironment can be included in the xenograft to more completely mimic the human tumor microenvironment
- Xenografts using NOD/SCID mice that have been ‘humanized’ by injection of peripheral blood or bone marrow cells, allow for an almost complete reconstitution of the immune response to the tumor

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Genetic engineered mouse models (GEMMs)

Application:

- Examination of the role of specific genes in tumor development and progression
- Evaluation of the effects of specific mutation, deletion or gene amplification of one or two genes during murine tumor progression
- Study of tumor progression over time

Advantages:

- Intact immune system
- Spontaneous tumor development
- Mice are immunocompetent, such that the tumor microenvironment can be mirrored as much as possible in a murine tumor model
- Specific genetic abnormalities that are present in human tumors can be reproduced, in an inducible manner, at specific ages in the tissue-type of origin
- Several therapeutic approaches can be explored at various stages of tumor development
- Applicable in humanized mice, where human genes, such as the cytochrome P450 genes or human tumor antigens, are expressed in mice to follow drug metabolism or immunological responses to the tumor

Return

Tools available from Celther

Celther Primary Human Cancer Cell Lines

CLTH/PC **Celther Number: CL 04001 -CLTH**	**Organism:** Homo sapiens, human **Organ:** Prostate **Disease:** Prostate cancer
CLTH/BC **Celther Number: CL 04002 -CLTH**	**Organism:** Homo sapiens, human **Organ:** Breast **Disease:** Breast cancer
CLTH/CC **Celther Number: CL 04003 -CLTH**	**Organism:** Homo sapiens, human **Organ:** Colon **Disease:** Colon cancer

All primary cell lines are in 1st-2nd passage. It is a mixed population of cells

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