Frontiers in Personalized Medicine, TEDD Symposium
June 18th 2015

Osteosarcoma: Current Perspectives and Future Developments

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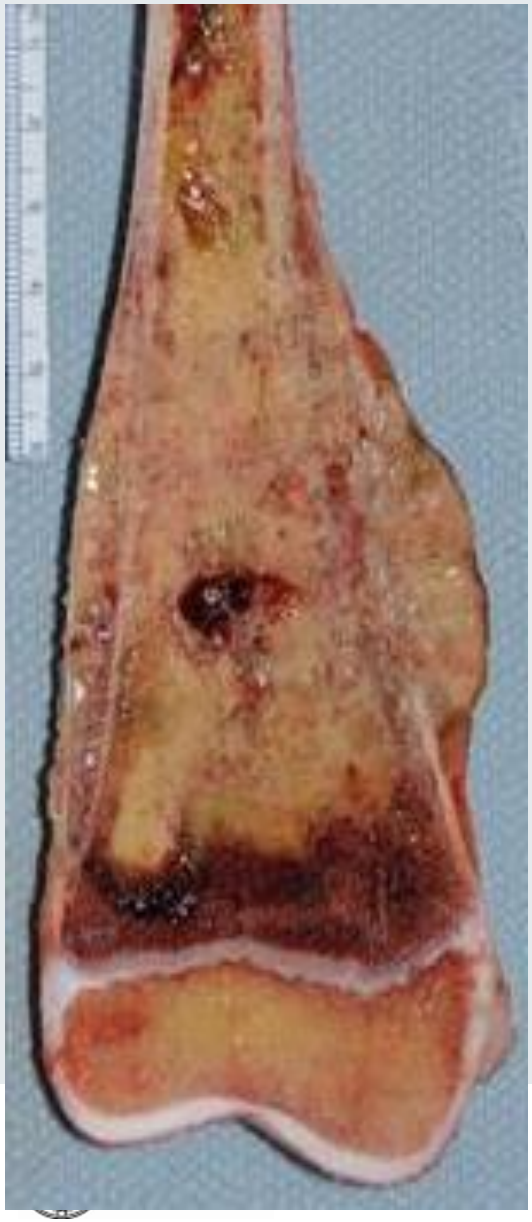
Sarkomzentrum ZÜRICH

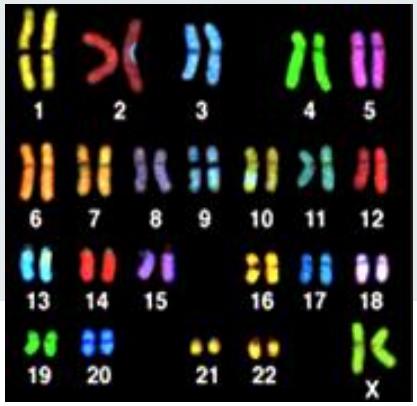
OSTEOSARCOMA

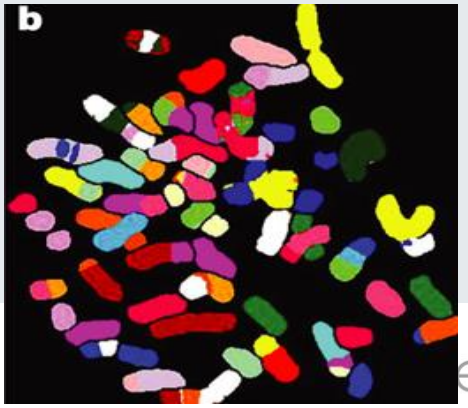


Definition :

- malignant spindle cells
- osteoid production

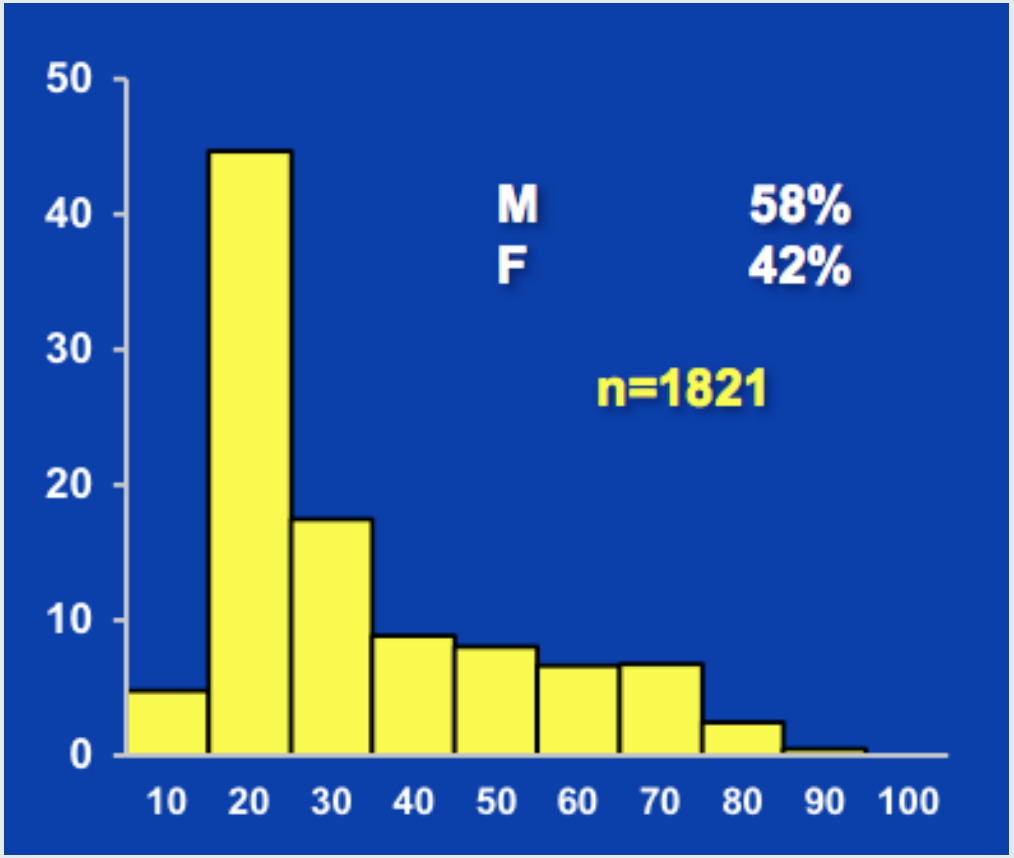
- most common 1° bone cancer
- - 2-3 / year per 1 M
- ~12 histologic subtypes

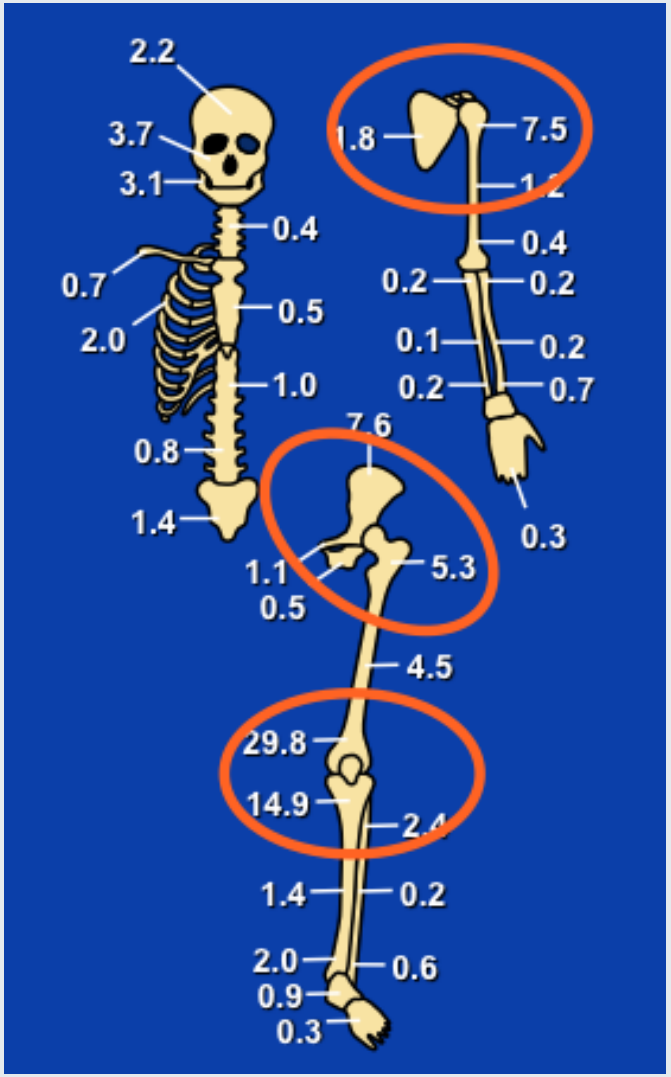




entrum
ZÜRICH

OSTEOSARCOMA

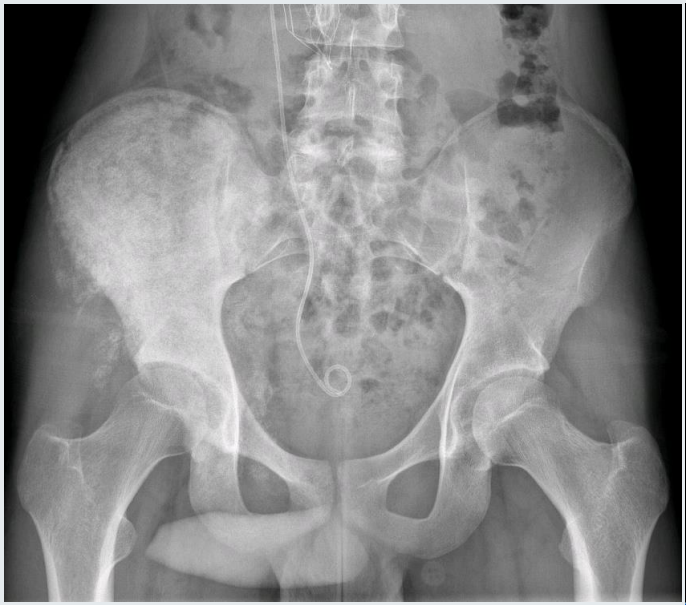


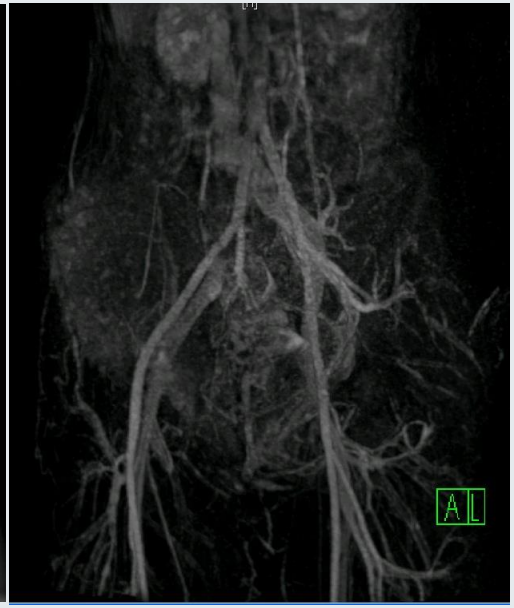


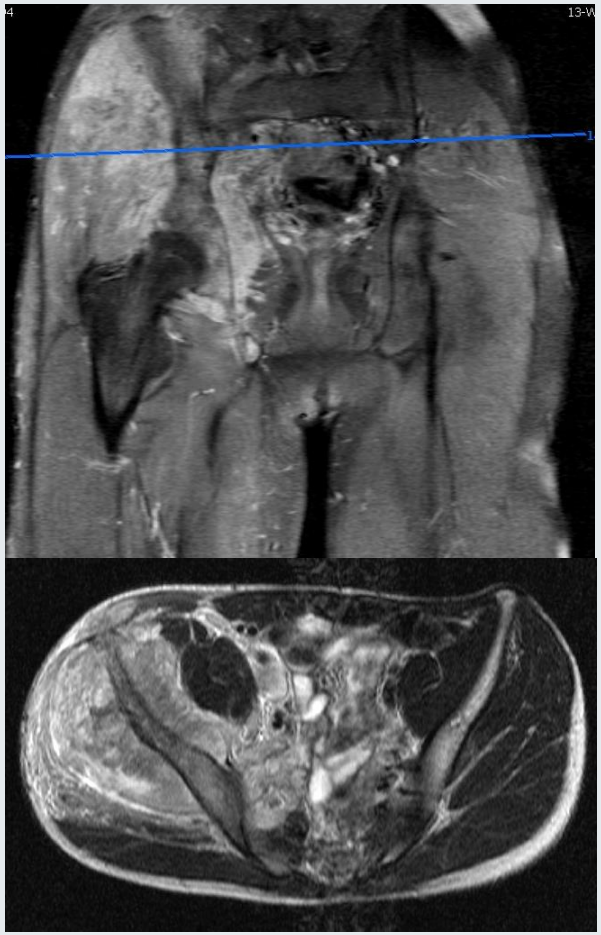
OSTEOSARCOMA
Backbone of Therapy

CASE PRESENTATION

16 year old young man

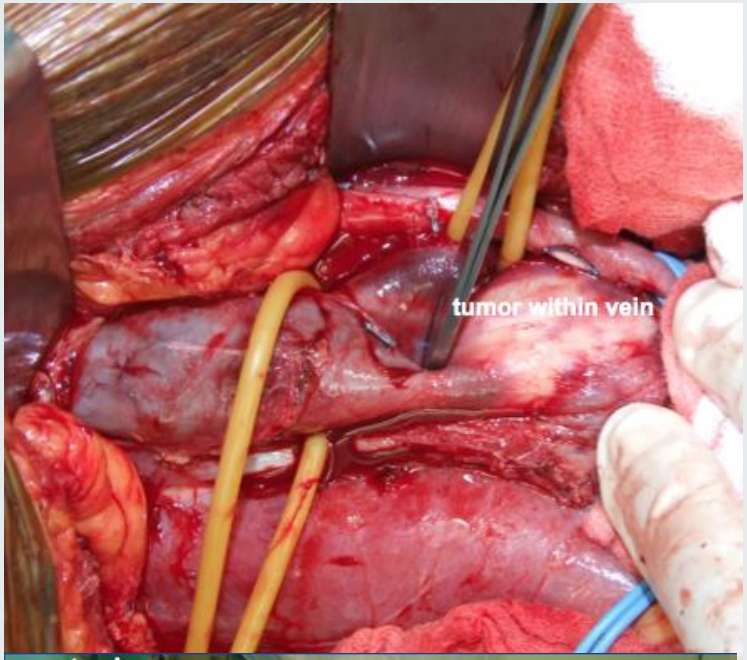


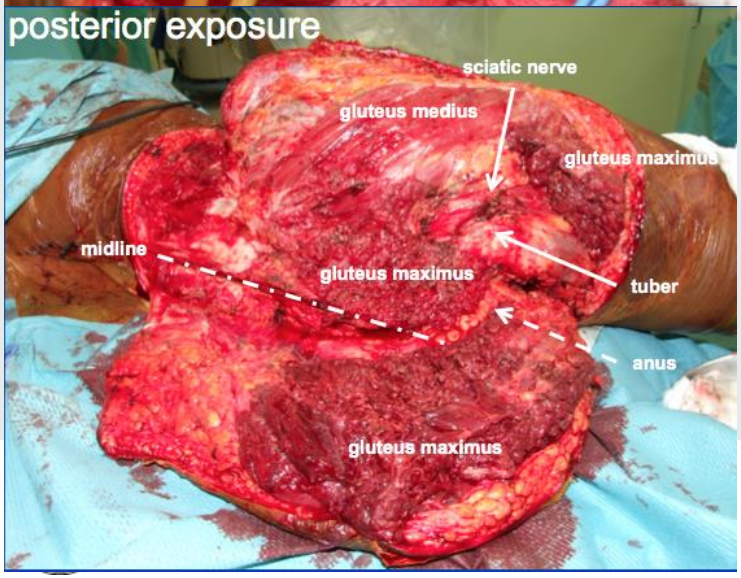


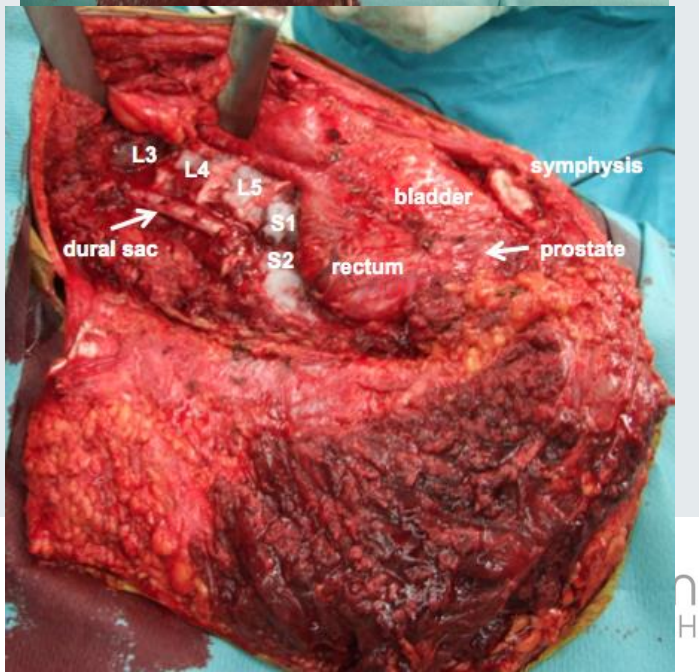


→ (neo-)adjuvant chemotherapy & surgery

CASE PRESENTATION







CASE PRESENTATION

Patient dies of metastasis

3 years post surgery

EURAMOS-1

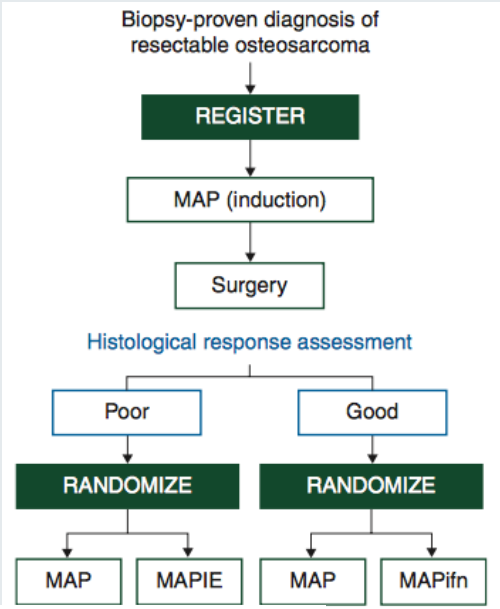
Annals of Oncology 26: 407–414, 2015
doi:10.1093/annonc/mdu526
Published online 24 November 2014

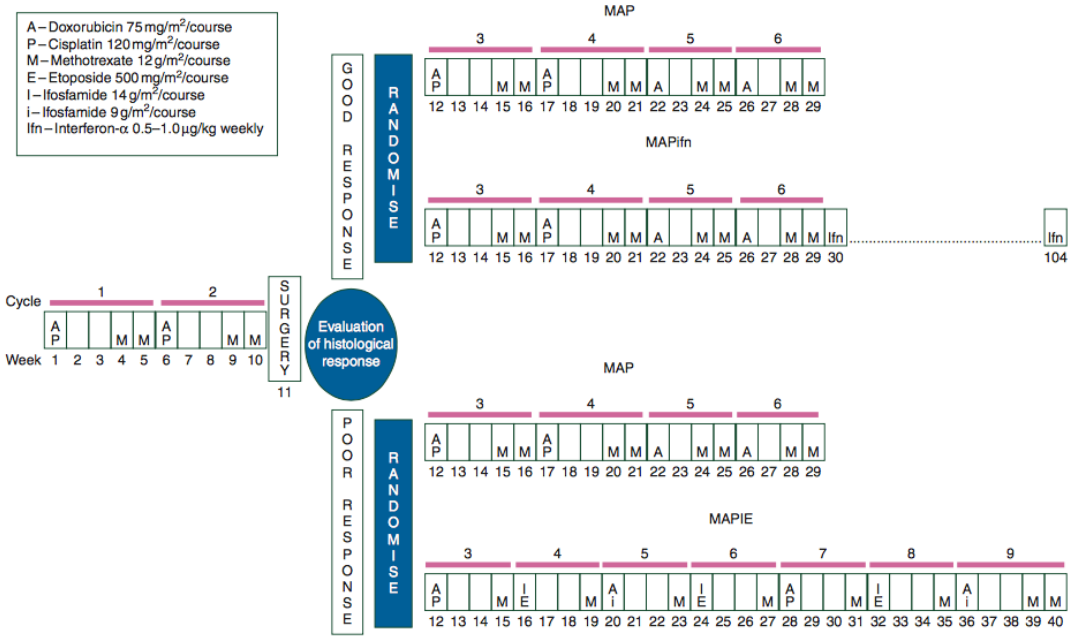
EURAMOS-1, an international randomised study for osteosarcoma: results from pre-randomisation treatment†

J. S. Whelan[1], S. S. Bielack[2], N. Marina[3], S. Smeland[4,5], G. Jovic[6], J. M. Hook[6], M. Krailo[7], J. Anninga[8], T. Butterfass-Bahloul[9], T. Böhling[10], G. Calaminus[11], M. Capra[12], C. Deffenbaugh[13], C. Dhooge[14], M. Eriksson[15], A. M. Flanagan[16,17], H. Gelderblom[8], A. Goorin[18], R. Gorlick[19], G. Gosheger[20], R. J. Grimer[21], K. S. Hall[22], K. Helmke[23], P. C. W. Hogendoorn[8], G. Jundt[24], [illegible][25], [illegible][26], [illegible]au[27], G. D. Letson[28], J. Meyer[29], P. A. Meyers[30], C. Morris[30,31], [illegible]ajan[34], R. L. Randall[35], P. Schomberg[36], R. Schwarz[37], L. A. Teot[38], [illegible][9],‡ on behalf of the EURAMOS collaborators§

Table 2. Pathology at diagnostic biopsy and surgery

	Diagnostic biopsy		Resected specimen	
	N	%	*N*	%
Data available				
Yes	2209	98	2012	89
No	51	2	248	11
Type of pathologist				
Reference	2160	98	1951	97
Local only	49	2	61	3
Histology				
Conventional	2033	92	1832	93
Telangiectatic	96	4	90	5
Small cell	14	1	7	0
High-grade surface	29	1	28	1
Secondary	0	0	0	0
Unclassified osteosarcoma	2	0	1	0
Ineligible	31	1	15	1
Not assessable	0	0	2	0
Info missing from the form	4	n/a	37	n/a
Excision of tumour				
Marginal	n/a	n/a	264	13
Wide	n/a	n/a	1474	74
Radical	n/a	n/a	222	11
Intra-lesional	n/a	n/a	26	1
Not known	n/a	n/a	19	1
Info missing from the form	n/a	n/a	7	n/a
Histological response				
Good (<10% viable tumour)	n/a	n/a	979	50
Poor (≥10% viable tumour)	n/a	n/a	996	50
Info missing from the form	n/a	n/a	37	n/a
Total	2209	100	2012	100





EURAMOS-1

Published Ahead of Print on June 8, 2015 as 10.1200/JCO.2014.60.0734
The latest version is at http://jco.ascopubs.org/cgi/doi/10.1200/JCO.2014.60.0734

JOURNAL OF CLINICAL ONCOLOGY | ORIGINAL REPORT

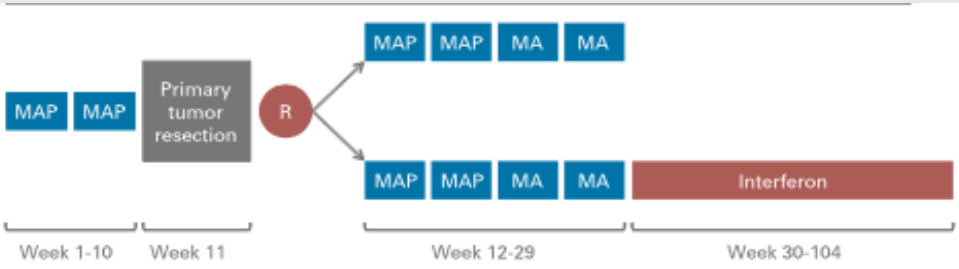
Methotrexate, Doxorubicin, and Cisplatin (MAP) Plus Maintenance Pegylated Interferon Alfa-2b Versus MAP Alone in Patients With Resectable High-Grade Osteosarcoma and Good Histologic Response to Preoperative MAP: First Results of the EURAMOS-1 Good Response Randomized Controlled Trial

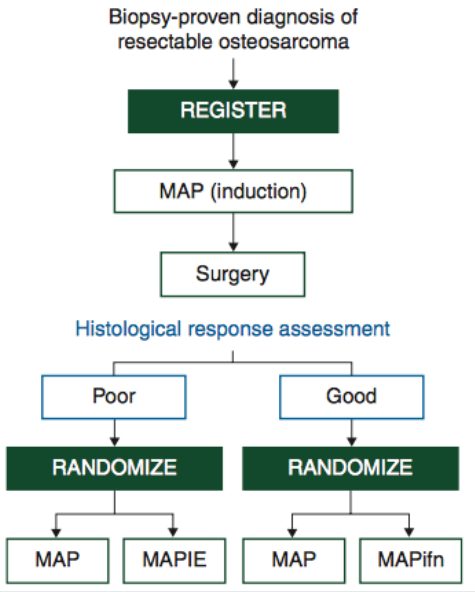
Stefan S. Bielack, Sigbjørn Smeland, Jeremy S. Whelan, Neyssa Marina, Gordana Jovic, Jane M. Hook, Mark D. Krailo, Mark Gebhardt, Zsuzsanna Pápai, James Meyer, Helen Nadel, R. Lor Randall, Claudia Deffenbaugh, Rajaram Nagarajan, Bernadette Brennan, G. Douglas Letson, Lisa A. Teot, Allen Goorin, Daniel Baumhoer, Leo Kager, Mathias Werner, Ching C. Lau, Kirsten Sundby Hall, Hans Gelderblom, Paul Meyers, Richard Gorlick, Reinhard Windhager, Knut Helmke, Mikael Eriksson, Peter M. Hoogerbrugge, Paula Schomberg, Per-Ulf Tunn, Thomas Kühne, Heribert Jürgens, Henk van den Berg, Tom Böhling, Susan Picton, Marleen Renard, Peter Reichardt, Joachim Gerss, Trude Butterfass-Bahloul, Carol Morris, Pancras C.W. Hogendoorn, Beatrice Seddon, Gabriele Calaminus, Maria Michelagnoli, Catharina Dhooge, Matthew R. Sydes, and Mark Bernstein, on behalf of the EURAMOS-1 investigators

Author affiliations appear at the end of this article.

Published online ahead of print at www.jco.org on June 1, 2015.

Support information appears at the end of this article.





A B S T R A C T

Purpose
EURAMOS-1, an international randomized controlled trial, investigated maintenance therapy with pegylated interferon alfa-2b (IFN-α-2b) in patients whose osteosarcoma showed good histologic response (good response) to induction chemotherapy.

Patients and Methods
At diagnosis, patients age ≤ 40 years with resectable high-grade osteosarcoma were registered. Eligibility after surgery for good response random assignment included ≥ two cycles of preoperative MAP (methotrexate, doxorubicin, and cisplatin), macroscopically complete surgery of primary tumor, < 10% viable tumor, and no disease progression. These patients were randomly assigned to four additional cycles MAP with or without IFN-α-2b (0.5 to 1.0 μg/kg per week subcutaneously, after chemotherapy until 2 years postregistration). Outcome measures were event-free survival (EFS; primary) and overall survival and toxicity (secondary).

Results
Good response was reported in 1,041 of 2,260 registered patients; 716 consented to random assignment (MAP, n = 359; MAP plus IFN-α-2b, n = 357), with baseline characteristics balanced by arm. A total of 271 of 357 started IFN-α-2b; 105 stopped early, and 38 continued to receive treatment at data freeze. Refusal and toxicity were the main reasons for never starting IFN-α-2b and for stopping prematurely, respectively. Median IFN-α-2b duration, if started, was 67 weeks. A total of 133 of 268 patients who started IFN-α-2b and provided toxicity information reported grade ≥ 3 toxicity during IFN-α-2b treatment. With median follow-up of 44 months, 3-year EFS for all 716 randomly assigned patients was 76% (95% CI, 72% to 79%); 174 EFS events were reported (MAP, n = 93; MAP plus IFN-α-2b, n = 81). Hazard ratio was 0.83 (95% CI, 0.61 to 1.12; $P = .214$) from an adjusted Cox model.

Conclusion
At the preplanned analysis time, MAP plus IFN-α-2b was not statistically different from MAP alone. A considerable proportion of patients never started IFN-α-2b or stopped prematurely. Long-term follow-up for events and survival continues.

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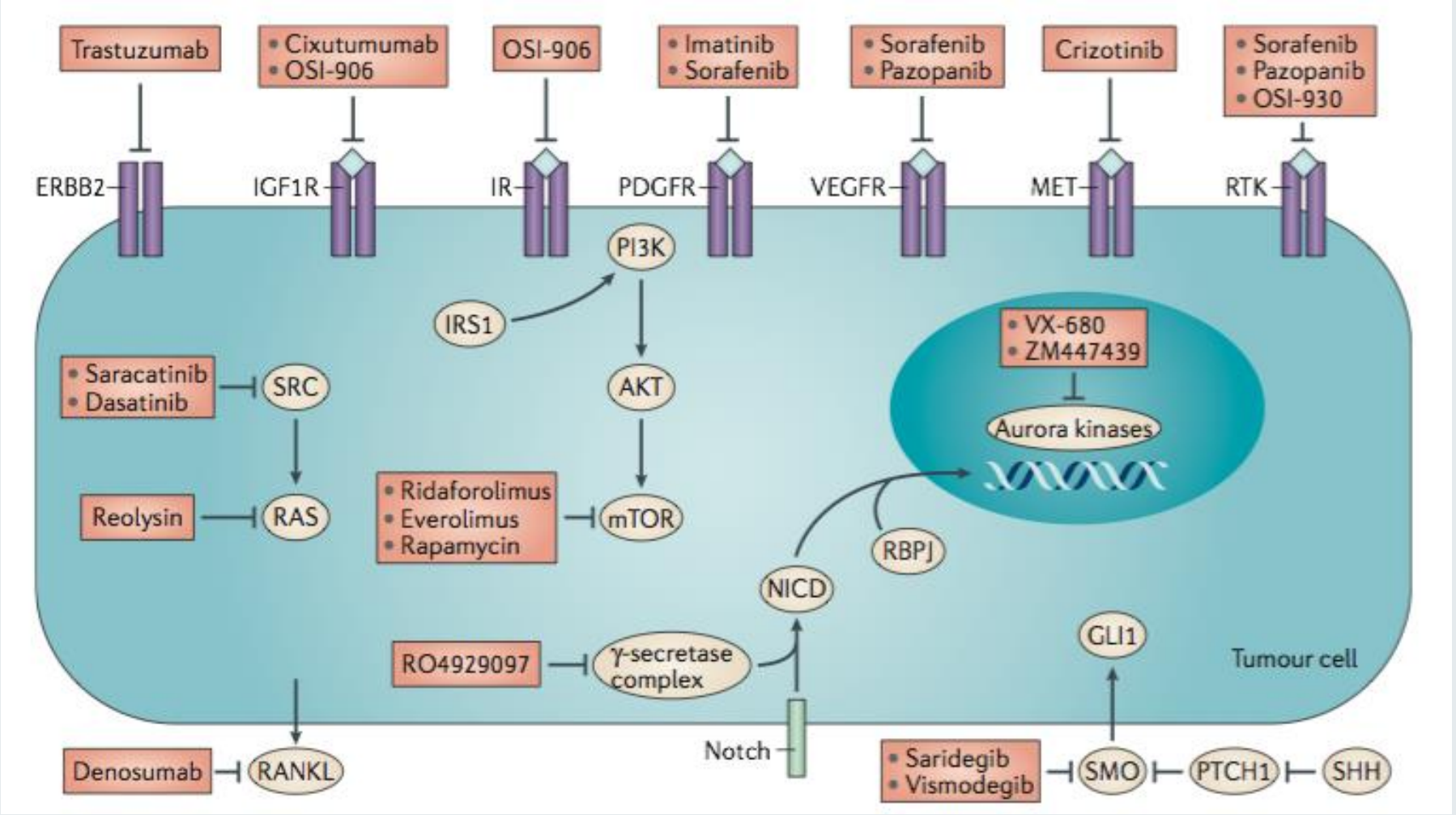
WHAT ELSE IS GOING ON ?

Advances in osteosarcoma therapy

The number of clinical trials testing substances or treatment methods against OS has increased over the last two decades [20•]. At present, there are 70 active clinical trials listed that include osteosarcoma patients, of which 21 trials are specifically aimed at targeting OS (see Supplementary Tables 1 and 2). The largest and most important ongoing OS trial is the EURAMOS-1 (EURopean and AMerican Osteosarcoma Studies, ClinicalTrials.gov identifier NCT00134030) trial, an inter-continental collaboration of 17 countries which started in 2005, with 2260

Botter S et al Cur Opinion Pharmacol 16:15-23, 2014

WHAT IS BEING TARGETED ?



Mansara M et al Nature Rev Cancer 14:722-35, 2014

Prof. S.Bielack on TARGETED THERAPY

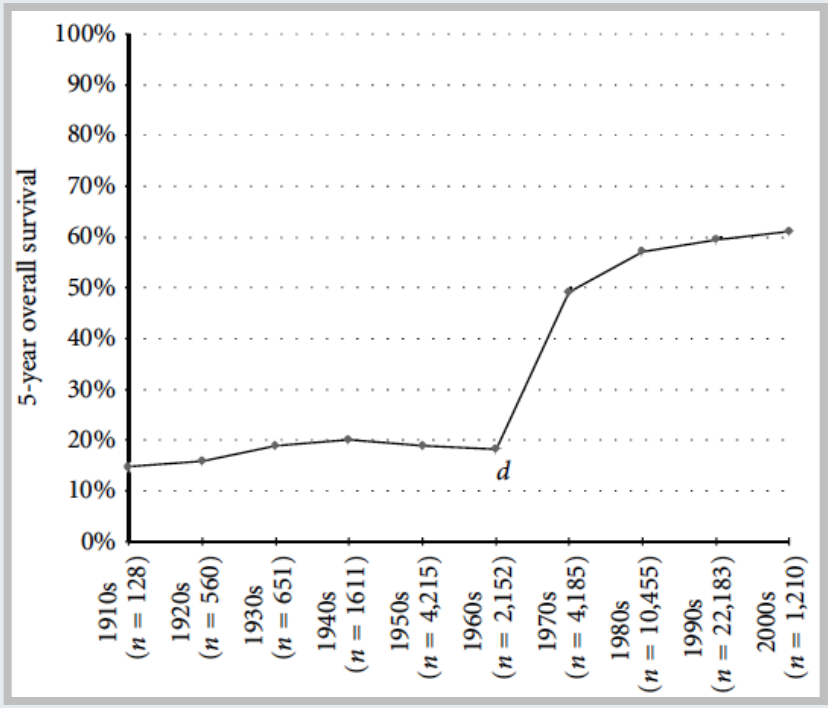
Personal Communication

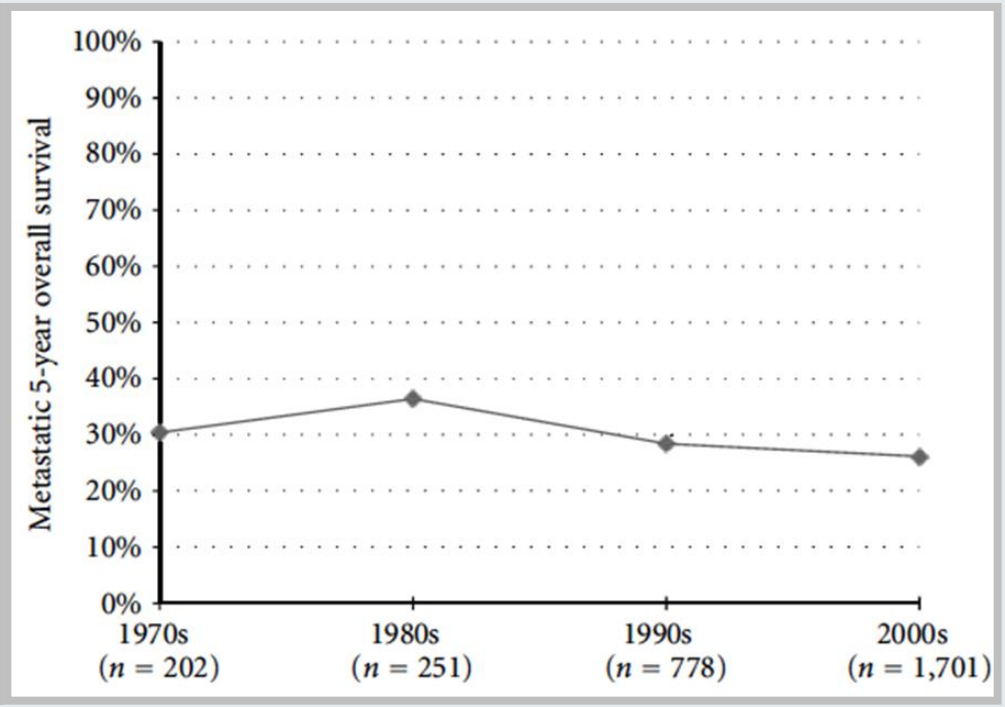
EMSOS Meeting, Athens, May 2015

- **we don't know what to target next !**
- **there is no further study planned**

the era of targeted therapy for OS is over !

OSTEOSARCOMA SURVIVAL





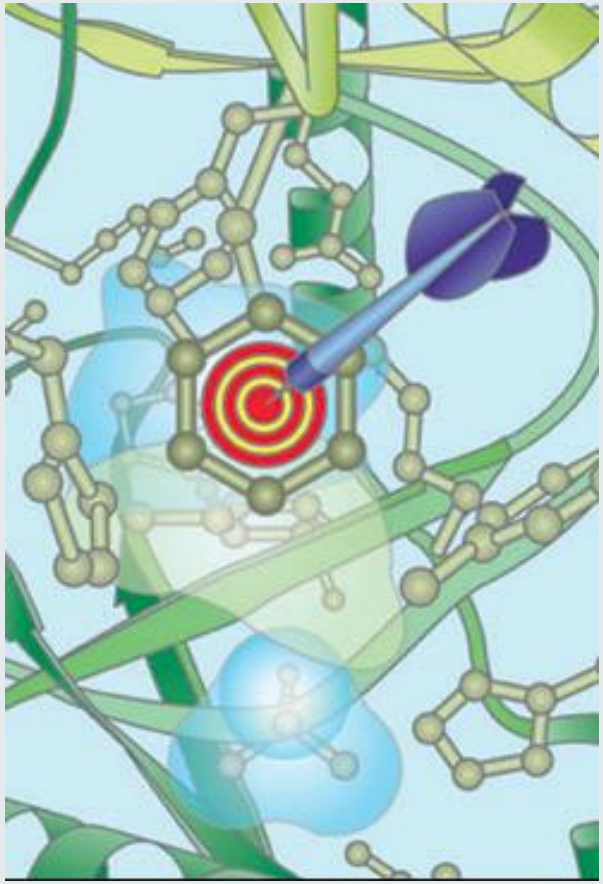
5yr overall survival

Metastasis

plateau, unchanged for years !

Allison D et al Sarcoma 2012, 2012, 704872

TARGETED THERAPY- BIOMARKER



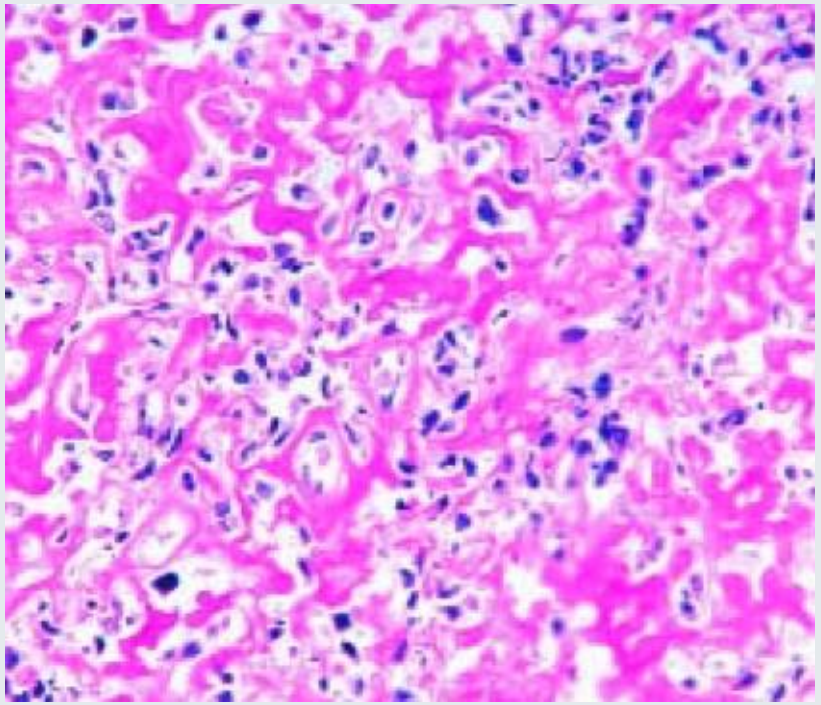
WHY DID IT FAIL ?

REASONS: HETEROGENEITY

Osteosarcoma Types

- Central
  - High-grade
    - Conventional
    - Telangiectatic
    - Small cell
    - Epithelioid
    - Osteoblastoma-like
    - Chondroblastoma-like
    - Fibrohistiocytic
    - Giant cell–rich
  - Low-grade
    - Low-grade central
      - Fibrous dysplasia–like
      - Desmoplastic fibroma–like
- Surface
  - Low-grade
    - Parosteal
  - Intermediate-grade
    - Periosteal
  - High-grade
    - Dedifferentiated parosteal
    - High-grade surface
- Intracortical
- Gnathic
- Extraskeletal
  - High-grade
  - Low-grade

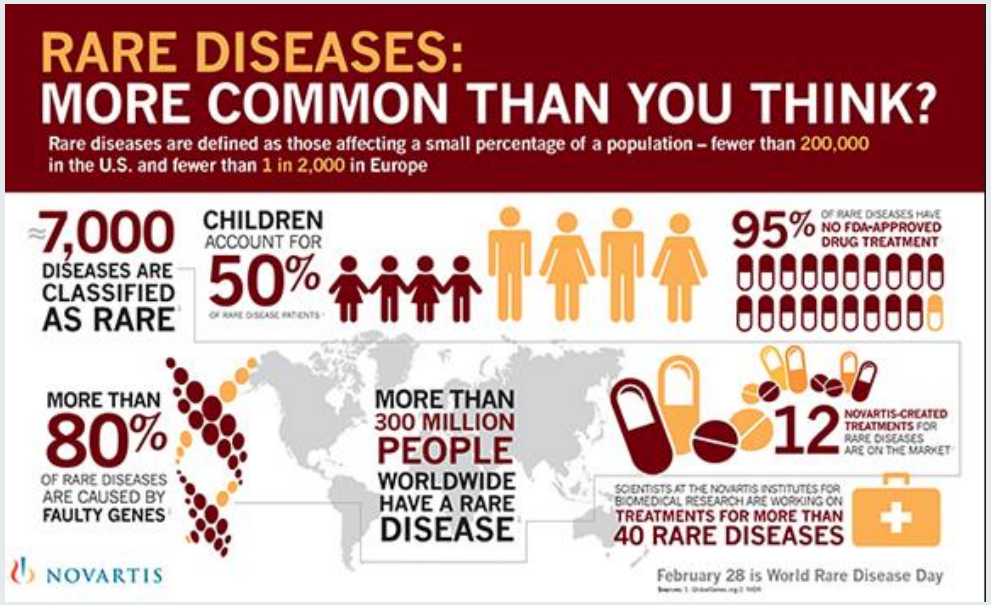
anatomically & histologically

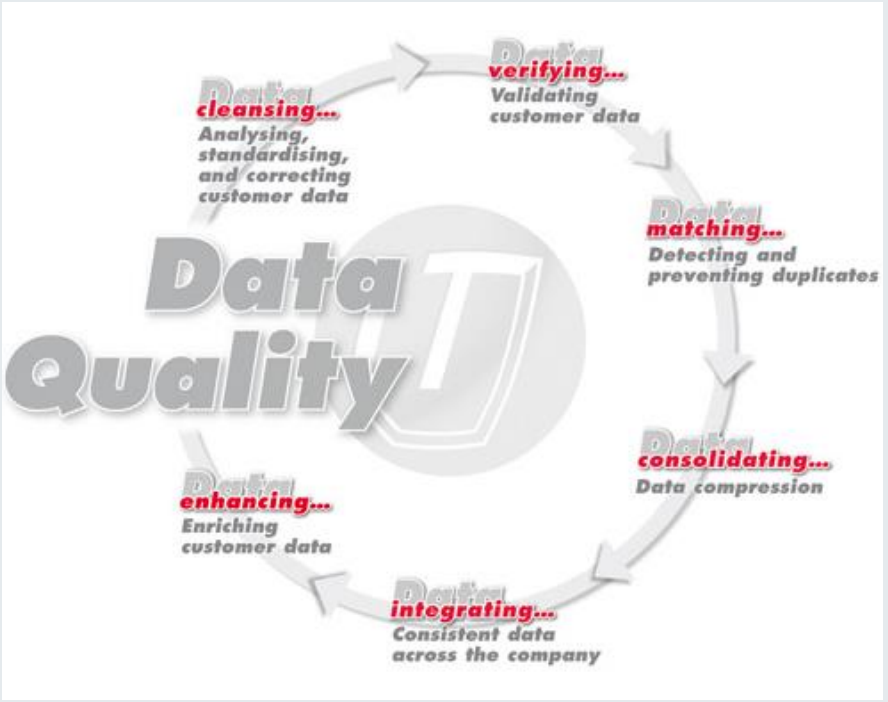


different subtypes of OS !

REASONS: DEMOGRAPHICS

Data Quality & Rarity of Disease





→ **biggest obstacles for progress !**

REASONS: HETEROGENEITY

Genome-wide analyses on high-grade osteosarcoma: Making sense of a genomically most unstable tumor

Marieke L. Kuijjer, Pancras C.W. Hogendoorn and Anne-Marie Cleton-Jansen

Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands

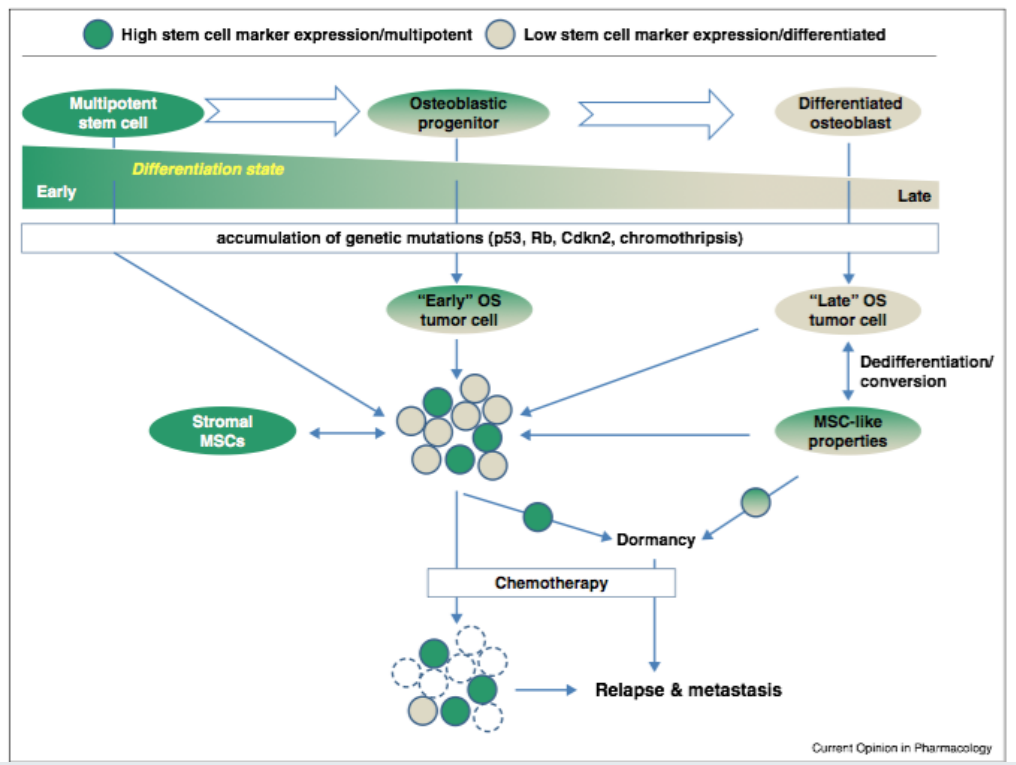
High-grade osteosarcoma is an extremely genomically unstable tumor. This, together with other challenges, such as the heterogeneity within and between tumor samples, and the rarity of the disease, renders it difficult to study this tumor on a genome-wide level. Now that most laboratories change from genome-wide microarray experiments to Next-Generation Sequencing it is important to discuss the lessons we have learned from microarray studies. In this review, we discuss the challenges of high-grade osteosarcoma data analysis. We give an overview of microarray studies that have been conducted so far on both osteosarcoma tissue samples and cell lines. We discuss recent findings from integration of different data types, which is particularly relevant in a tumor with such a complex genomic profile. Finally, we elaborate on the translation of results obtained with bioinformatics into functional studies, which has lead to valuable findings, especially when keeping in mind that no new therapies with a significant impact on survival have been developed in the past decades.

Analysis	Data type	Study	Osteosarcoma samples	Comparison	Pathway/genes
Single-way	*mRNA*	Kuijjer *et al.*[28]	76 B, 13 X, 18 C	Histological subtypes	NFκB in fibroblastic, chondroid-matrix associated genes in chondroblastic osteosarcoma. Primary tumor expression signatures are preserved in model systems
		Buddingh *et al.*[31]	53 B	Metastasis-free survival (MFS)	Macrophage-associated genes correlate with better MFS
		Su *et al.*[32]	3 C, 5 X	Capacity to metastasize	*IGFBP5* downregulation correlates with metastasis
		Namlos *et al.*[34]	12 B/T, 11 M	Tumor sample type	Immunological processes and chemokine pattern upregulated in metastases
		Cleton-Jansen *et al.*[33] Kuijjer *et al.*[28]	25 B 69 B	Response to chemotherapy	No significant differential expression
		Cleton-Jansen *et al.*[33]	25 B	Control samples (osteoblastoma, MSC, osteoblast)	Cell-cycle regulation, DNA replication pathways
		Sadikovic *et al.*[43]	6 B	Control sample (osteoblast)	DNA replication network
		Kuijjer *et al.*[44]	84 B	Control samples (MSC, osteoblast)	Apoptosis, signal transduction
		Kansara *et al.*[45]	5 C	Treatment with demethylating agent	*WIF1* methylation and downregulation
	miRNA	Jones *et al.*[46]	18 B	Control samples (normal bone)	miR-16 Downregulation, miR-27a association with metastasis
	CN	Kresse *et al.*[29]	9 T/M and their derived xenografts	Tumor sample type	Xenografts are representative for primary tumors although some additional aberrations are observed
		Squire *et al.*[51] Man *et al.*[52] Atiye *et al.*[53] Yang *et al.*[54] Kresse *et al.*[55] Kuijjer *et al.*[44] Lockwood *et al.*[56] Yen *et al.*[57] Smida *et al.*[59] Pasic *et al.*[60]	9 B 48 B/T/M 22 C/TS/R 20 B 36 TS/M/X, 20 C 32 B 22 TS 42 TS/R/M/C 45 B 27 B	Control samples	Overall high level of aneuploidy, which seems non-random. Regions described by three or more studies are gains on 1p, 6p, 8q, 12q and 17p and losses on 2q, 3q, 6q, 10, 13q and 17p
		Kuijjer *et al.*[44] Smida *et al.*[59]	32 B 45 B	Metastasis/event-free survival	Genomic alterations are prognostic predictors
		Yen *et al.*[57]	23 TS, 14 R/M	Tumor sample type	Identified deletions/amplifications which differ between TS and R/M
		Kresse *et al.*[55] Yen *et al.*[57] Pasic *et al.*[60]	36 TS/M/X, 20 C 42 TS/R/M/C 27 B	Control samples	Frequent deletion of *LSAMP*
		Yang *et al.*[54]	20 B	Control samples	Enrichment of VEGF pathway
Integrative	*CN, mRNA*	Kuijjer *et al.*[44]	29 B	Control samples (MSC, osteoblast)	Set of 31 candidate drivers enriched in genes with a role in genomic instability
		Lockwood *et al.*[56]	22 TS, 8 X	Control samples (normal tissues)	Amplification and overexpression of cyclin E3
	miRNA, mRNA	Jones *et al.*[46]	14 B	Control samples (normal bone)	Transcriptional regulation, cell-cycle control and cancer signaling
		Namlos *et al.*[47]	19 C	Control samples (normal bone)	Pairs of miRNAs with 26 mRNAs

aberrant karyotypes / chromotripsis !

REASONS - CELL OF ORIGIN

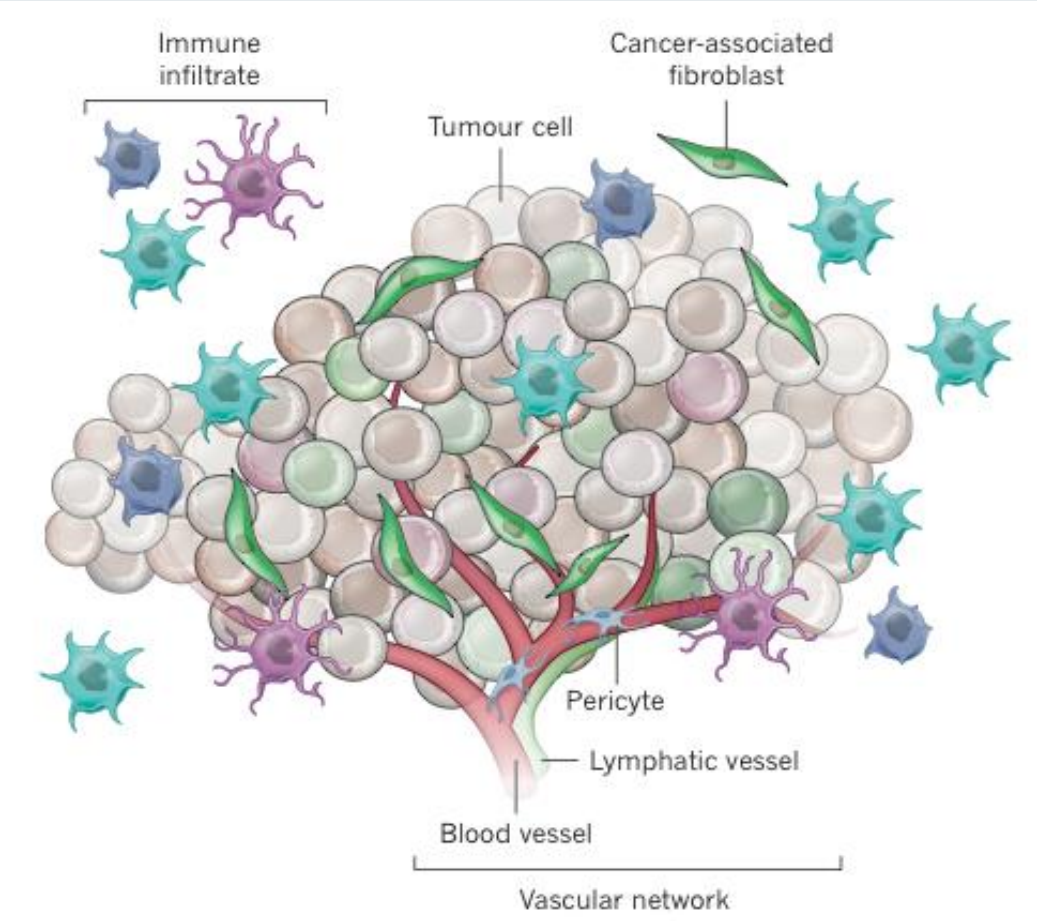
what is the cell of origin ?

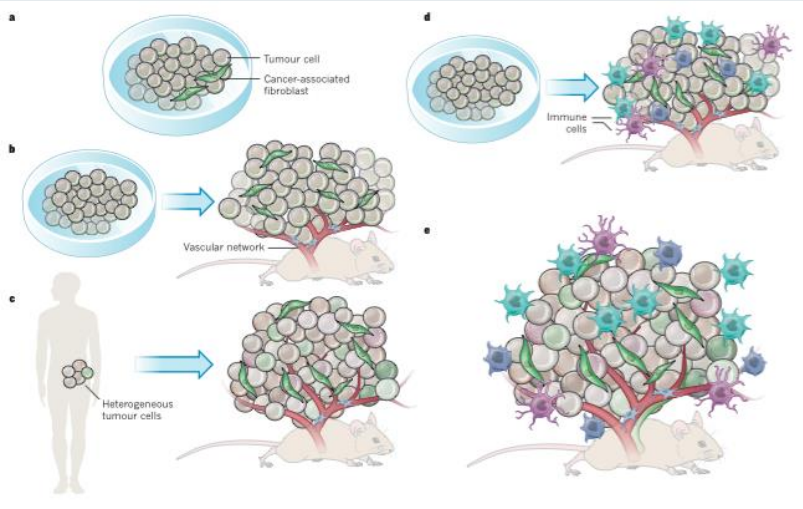


If it was possible to target cell of origin, then we could improve OS survival !

Botter S et al Cur Opinion Pharmacol 16:15-23, 2014

REASONS: MICROENVIRONMENT





Challenge of modelling microenvironment

tumor environment / vascular network / host's immune system!

Junttila M et al Nature 501:346-54, 2013

REASONS: MODEL SYSTEMS
in vitro & in-vivo models

PLOS ONE | DOI:10.1371/journal.pone.0125611 May 19, 2015

RESEARCH ARTICLE

Genomic Instability of Osteosarcoma Cell Lines in Culture: Impact on the Prediction of Metastasis Relevant Genes

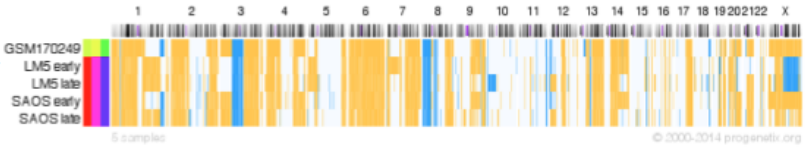
Roman Muff[1], Prisni Rath[2], Ram Mohan Ram Kumar[1], Knut Husmann[1], Walter Born[1], Michael Baudis[2], Bruno Fuchs[1]*

1 Laboratory for Orthopedic Research, Balgrist University Hospital, Zurich, Switzerland, **2** Institute of Molecular Life Sciences, University of Zurich, Zurich, Switzerland

Conclusions

Considerable instability during culture in terms of gene expression and chromosomal aberrations was observed in osteosarcoma cell lines. The use of cells from different passages and a search for genes consistently regulated in early and late passages allows the analysis of metastasis-relevant genes despite the observed instability in gene expression in osteosarcoma cell lines during culture.

A



B

	Total	Gain	Loss	Gain/Loss
	%			
GSM170249	51.5	45.8	5.7	8.0
SAOS early	48.1	43.2	4.9	8.8
SAOS late	45.3	40.1	5.2	7.7
LM5 early	46.6	39.8	6.8	5.9
LM5 late	45.9	38.0	7.9	4.8

C

	SAOS early	SAOS late	LM5 early	LM5 late
	%			
SAOS early	0	12.1	15.3	20.7
SAOS late		0	20	18.2
LM5 early			0	10.8
LM5 late				0

→ cell lines are unstable !

Muff R et al. PlosOne DOI:10.1371; 2015

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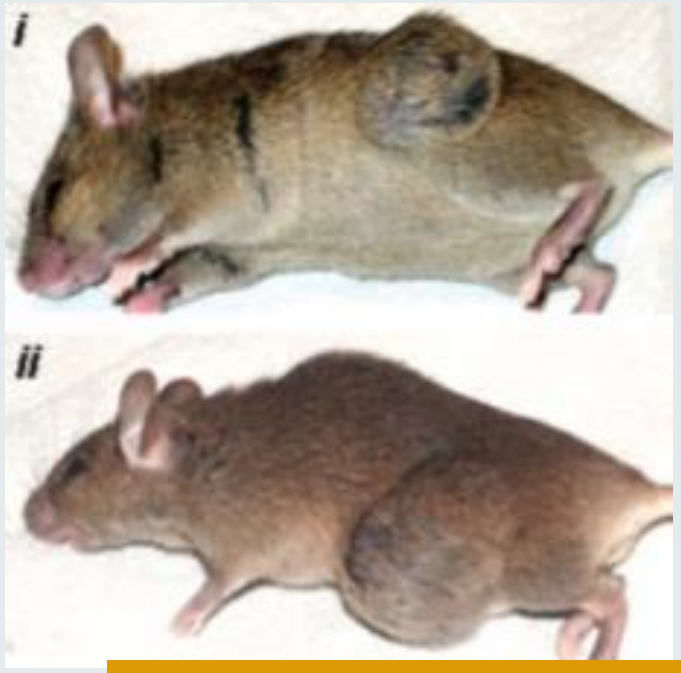
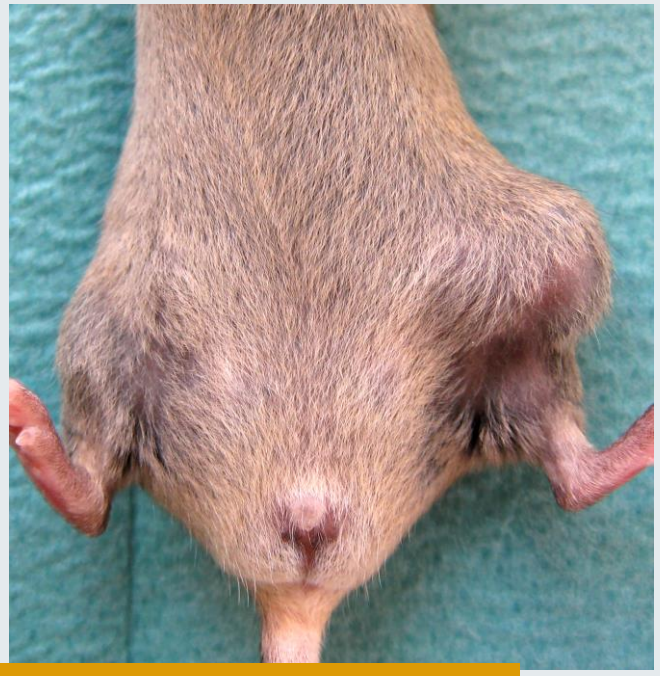
REASONS: MODEL SYSTEMS
in vitro & in-vivo models

sc injection



it injection



same cells, injection at different locations
→ different metastatic pattern!

→ imperfect in-vivo model systems !

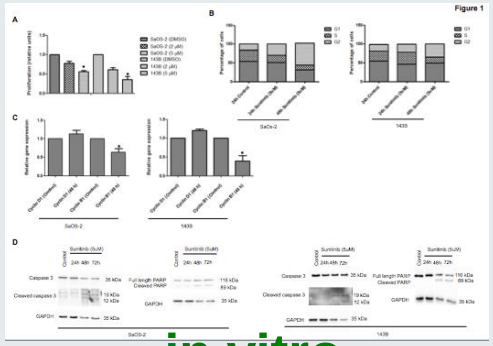
REASONS: APPROACH ?

Mono- / Combination Therapy

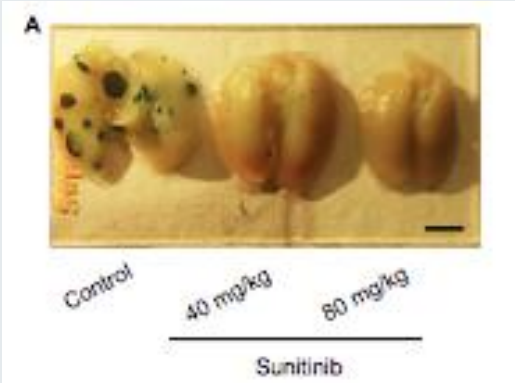
Sunitinib malate (SU-11248) reduces tumour burden and lung metastasis in an intratibial human xenograft osteosarcoma mouse model

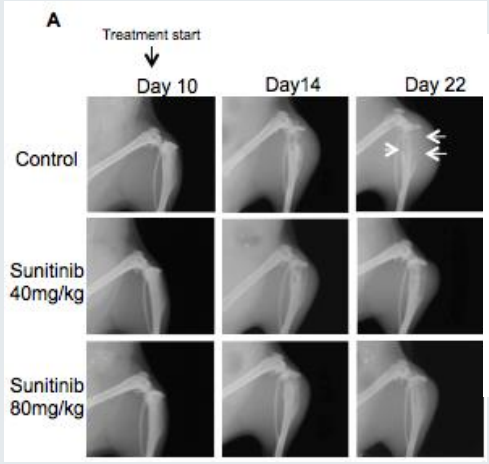
Ram Mohan Ram Kumar*[a], Matthias J.E. Arlt[a], Bernhard Robl[a], Aleksandar Kuzmanov[a], Walter Born[a], Bruno Fuchs[a]

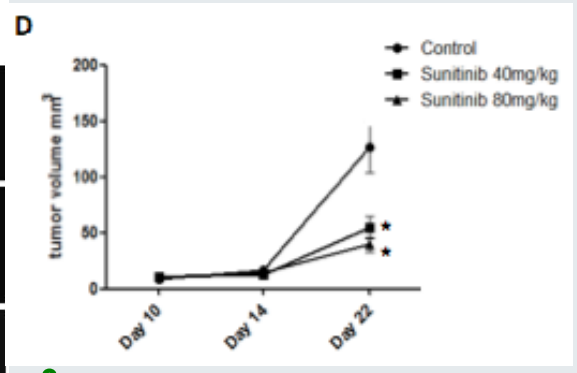
[a]Laboratory for Orthopaedic Research, Department of Orthopaedics, Balgrist University Hospital, University of Zurich, Zurich, Switzerland



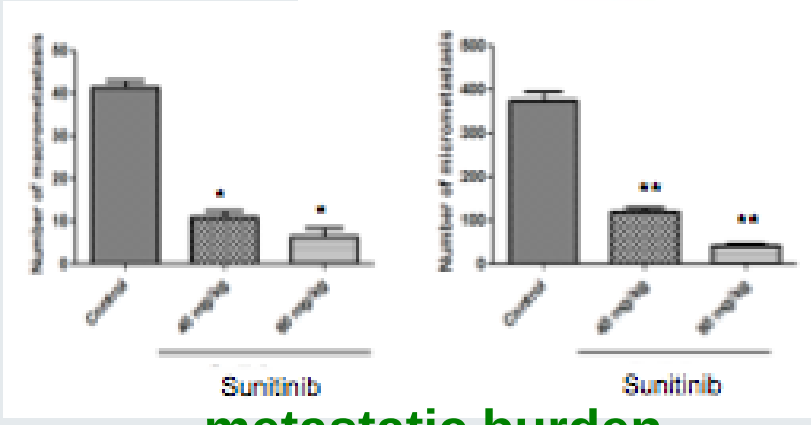
in vitro







1° Tumor



metastatic burden

Mohan R et al. Am J Cancer Res in publication

Universität Zürich[UZH]

Sarkomzentrum ZÜRICH

WHAT IS BEING TARGETED ?

Translational biology of osteosarcoma

Maya Kansara[1,2], Michele W. Teng[3,4], Mark J. Smyth[3,4] and David M. Thomas[1,2,5]

Abstract | For the past 30 years, improvements in the survival of patients with osteosarcoma have been mostly incremental. Despite evidence of genomic instability and a high frequency of chromothripsis and kataegis, osteosarcomas carry few recurrent targetable mutations, and trials of targeted agents have been generally disappointing. Bone has a highly specialized immune environment and many immune signalling pathways are important in bone homeostasis. The success of the innate immune stimulant mifamurtide in the adjuvant treatment of non-metastatic osteosarcoma suggests that newer immune-based treatments, such as immune checkpoint inhibitors, may substantially improve disease outcome.

→ personalized medicine !

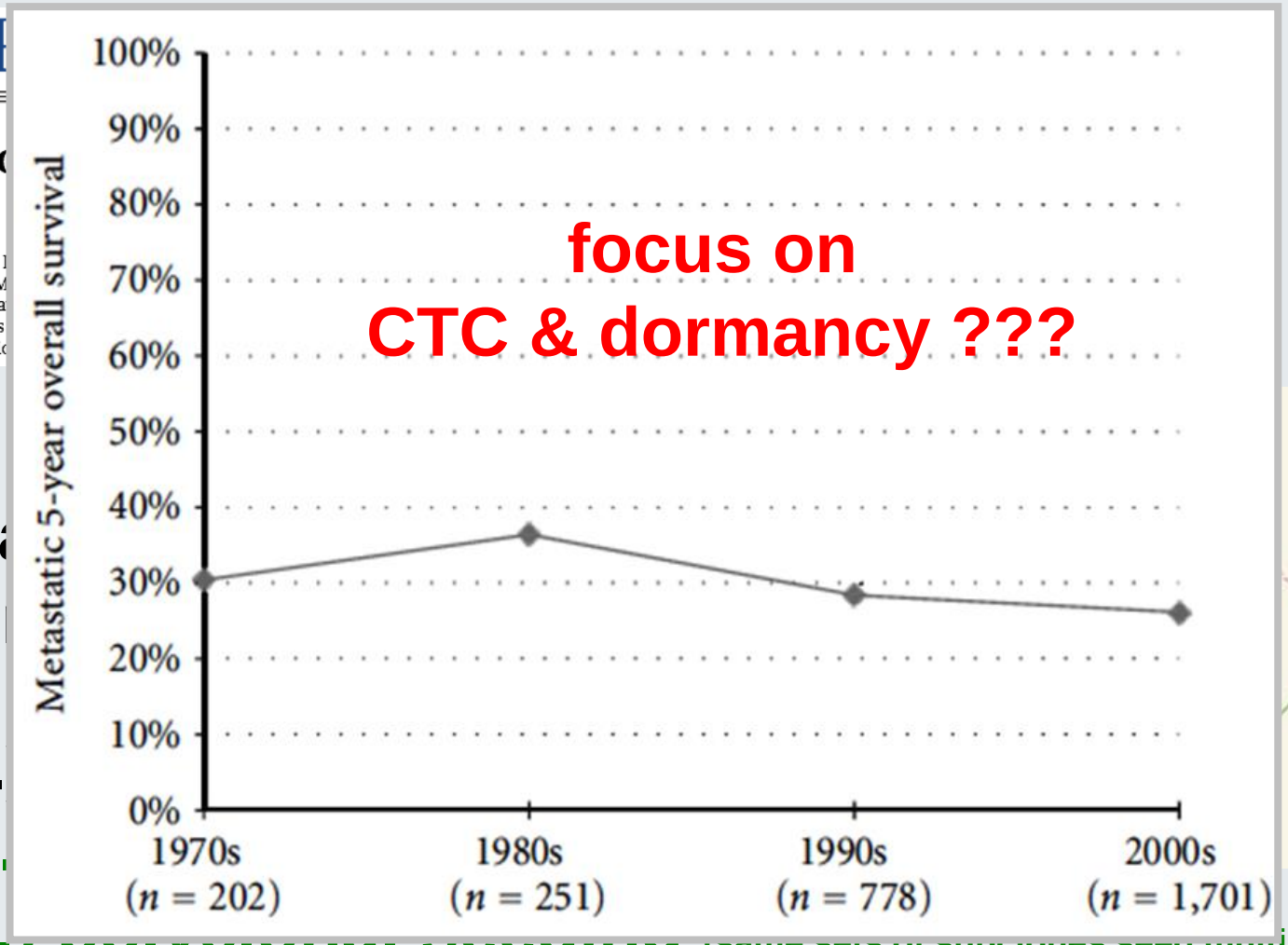
Mansara M et al Nature Rev Cancer 14:722-35, 2014

LATEST DEVELOPMENTS

LETTE

The evolutio
cancer

Gunes Gundem[1], Peter Van
Daniel S. Brewer[4,5], Heini M
Sarah O'Meara[1], Kevin J. Da
Zsofia Kote-Jarai[10], Douglas
Rosalind A. Eeles[10,14]§, Tapio



Metasta
subclo

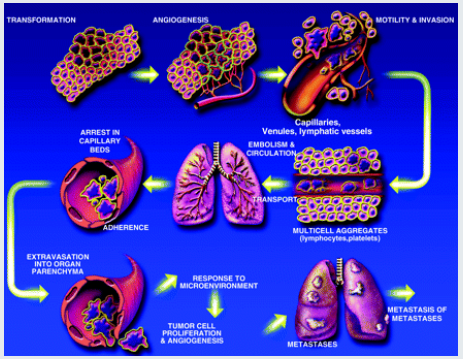
a.

b.

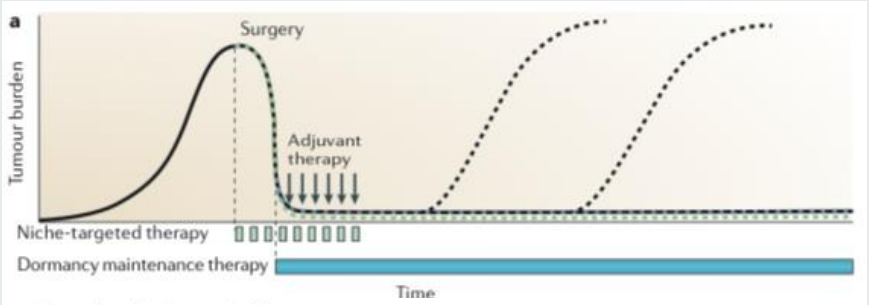
c.) polyclonal seeding (same sets of subclones seed multiple sites)

LATEST DEVELOPMENTS

CTC & Dormancy



Nature Reviews Cancer (15):239-247, 2015



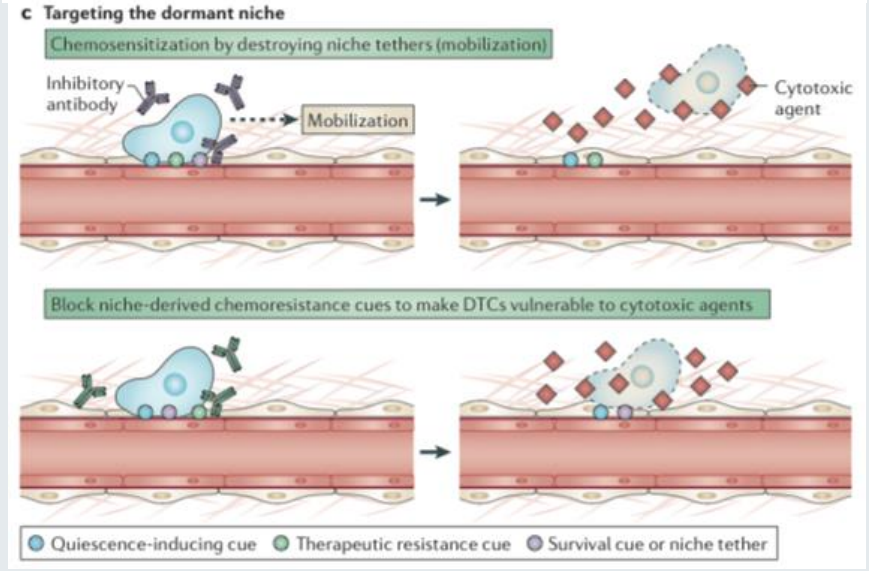
PERSPECTIVES

OPINION

Metastasis prevention by targeting the dormant niche

Cyrus M. Ghajar

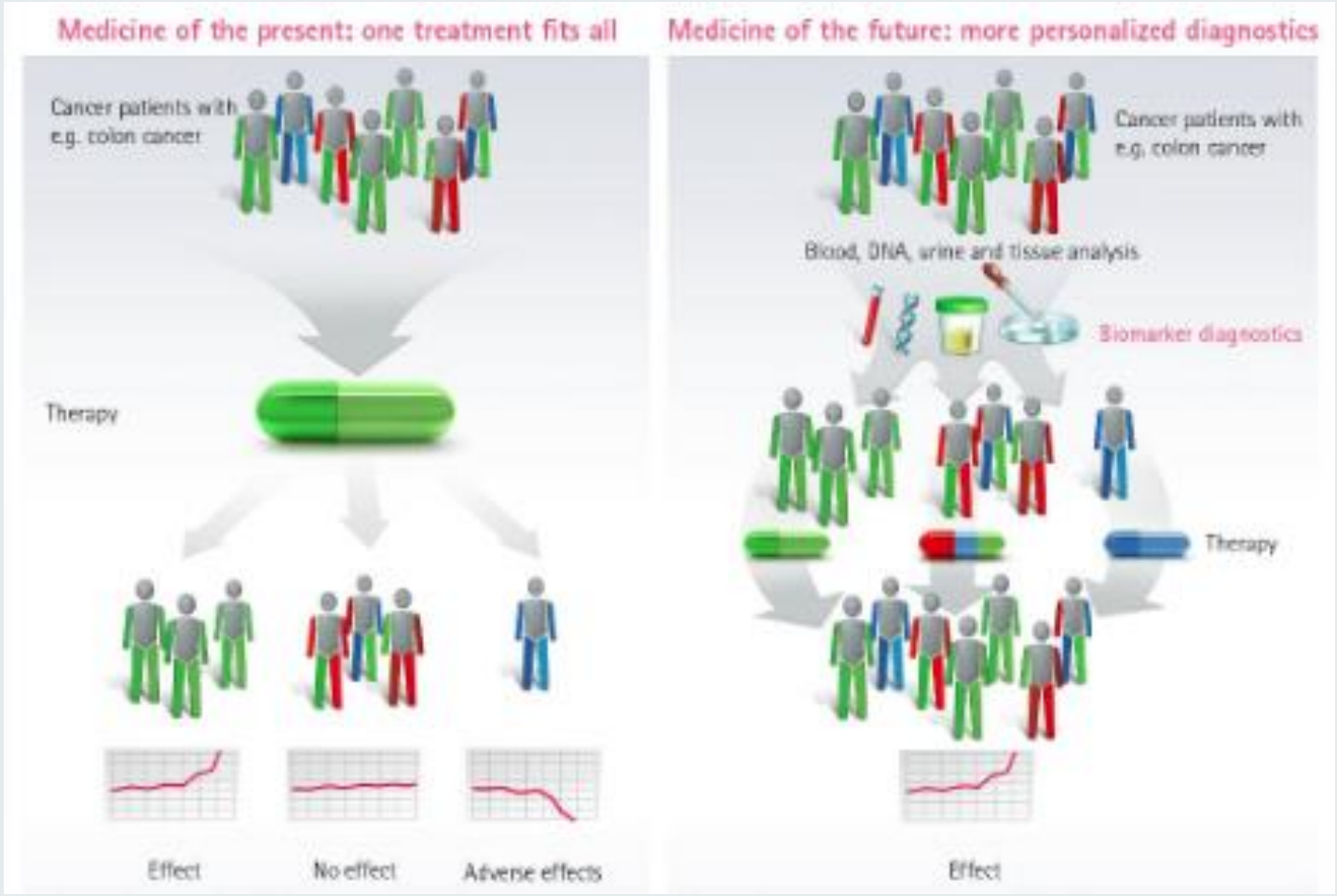
Abstract | Despite considerable advancements that shattered previously held dogmas about the metastatic cascade, the evolution of therapies to treat metastatic disease has not kept up. In this Opinion article, I argue that, rather than waiting for metastases to emerge before initiating treatment, it would be more effective to target metastatic seeds before they sprout. Specifically, I advocate



1° TU cells → disseminated tumor cells

PERSONALIZED MEDICINE

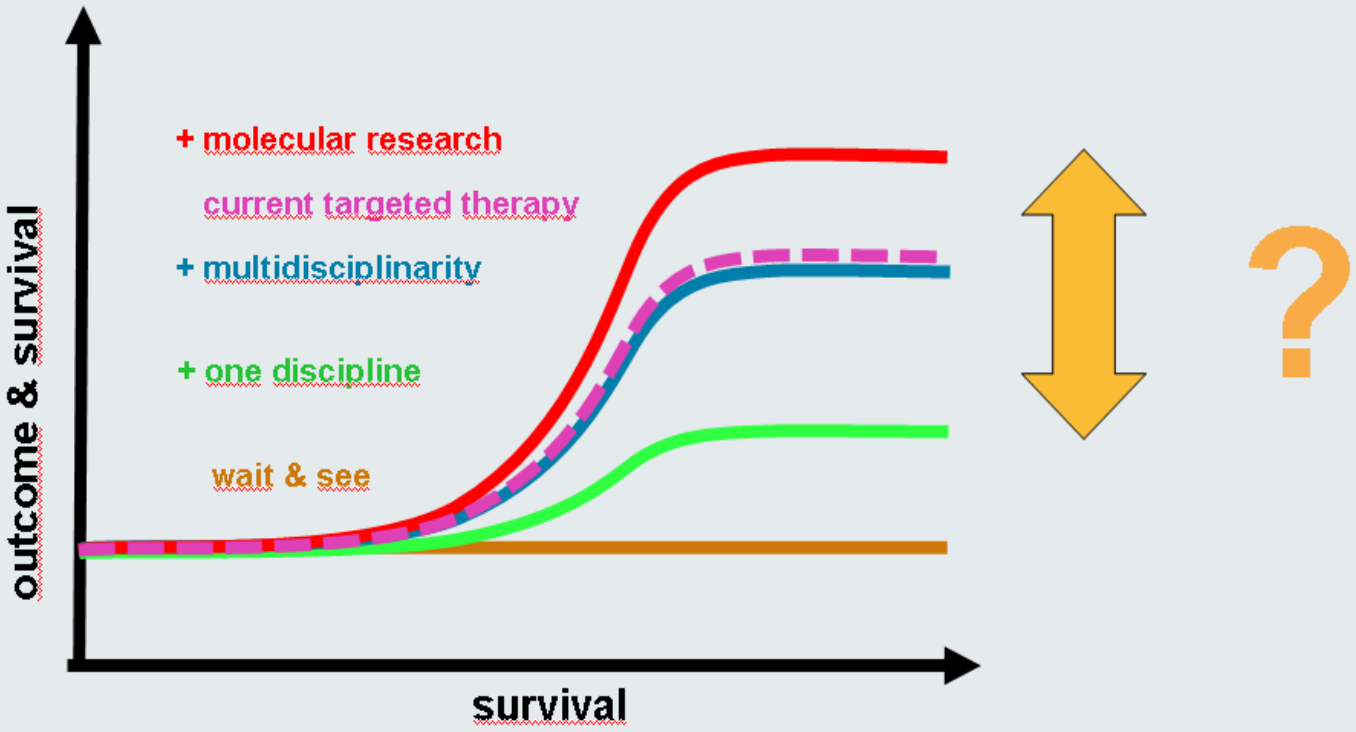
Tailored Treatments



→ it‘s getting more intense !

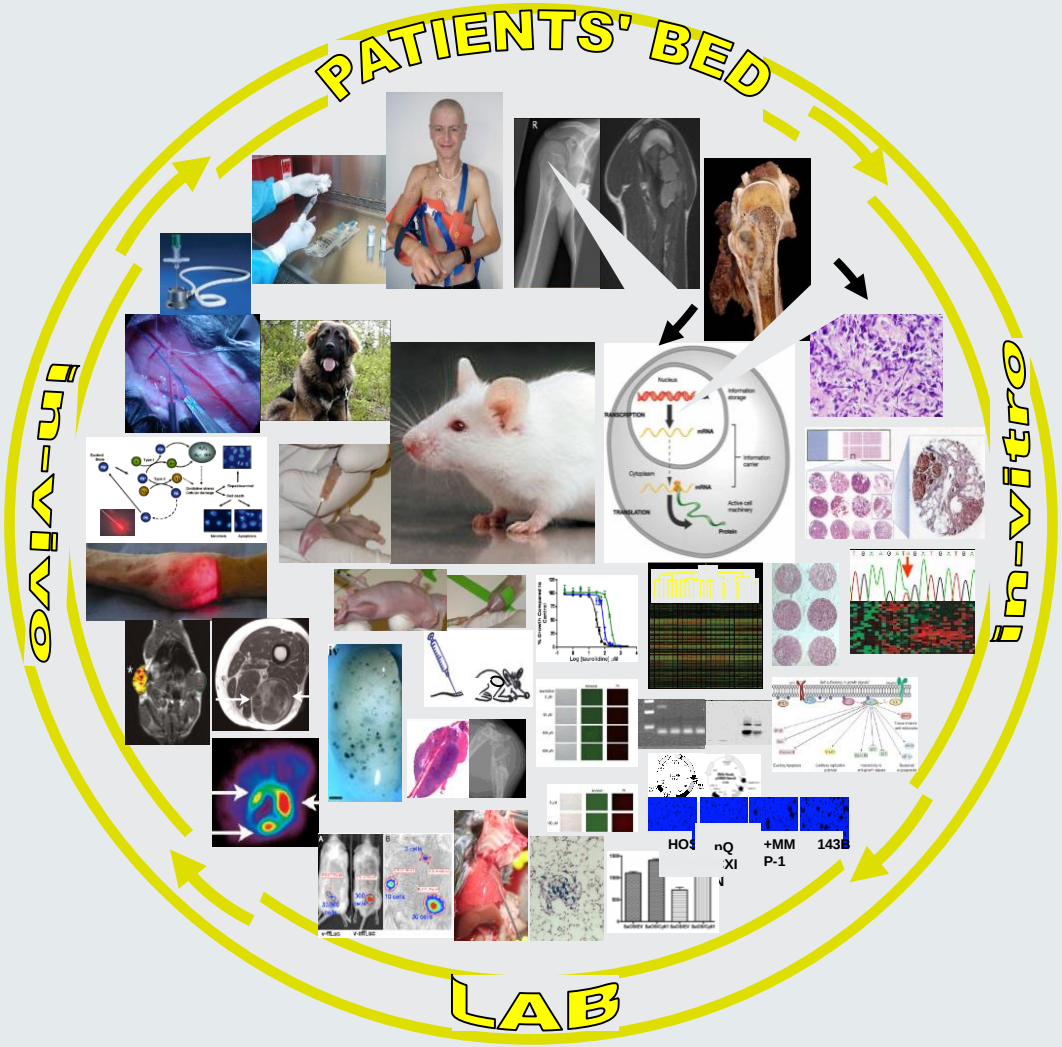
PERSONALIZED MEDICINE

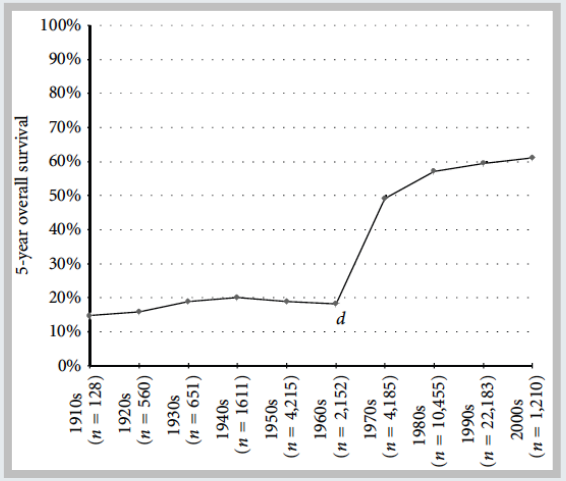
what do we want ?



→ how do we invest ?

QUEST FOR PERSONALIZED MEDICINE





diagnostic markers

improved imaging

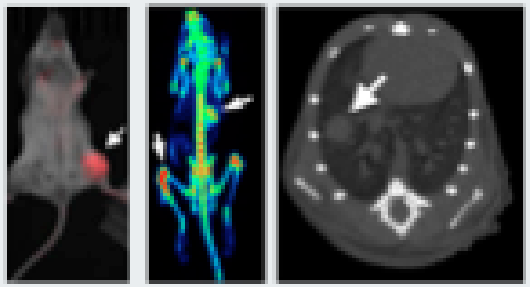
individualized therapy

→ prognosis ↑

QUESTS FOR PERSONALIZED MEDICINE

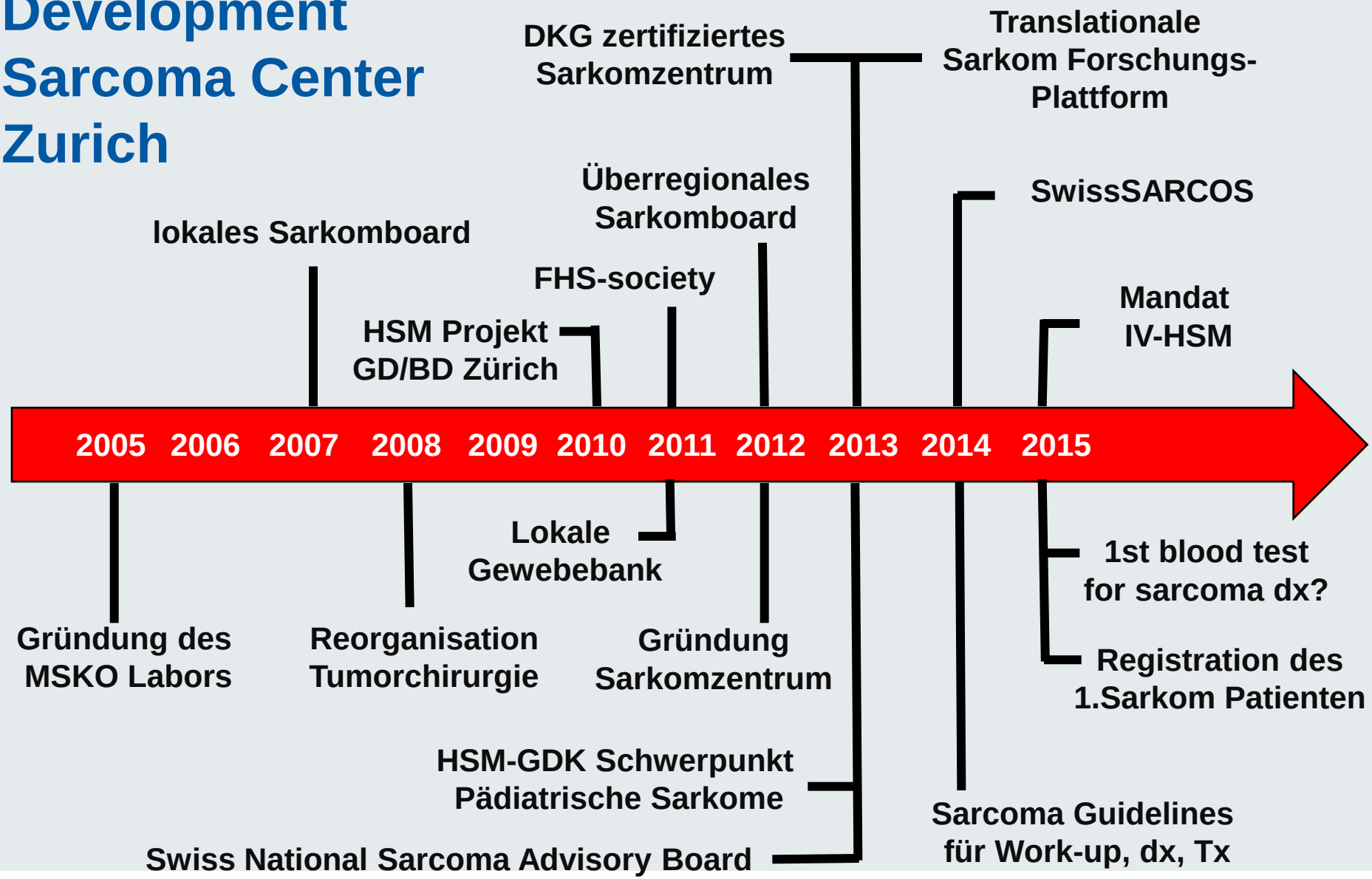
3 Major Areas

Cooperation Clinical Data	Patient material based research	therapy/diagnostics Imaging/monitoring
Local, national and international level	- Patient tissue xenograft (PDX) models - Drug testing	Quantitative treatment monitoring (experimental, clinical)



Overall goal: Improve sarcoma patient survival through development of novel, patient-tailored therapies

Development Sarcoma Center Zurich



Swiss National Sarcoma Advisory Board

A. **Definition of Advisory Board / Sarcoma Boards / Sarcoma Centers**

B. **Guidelines**

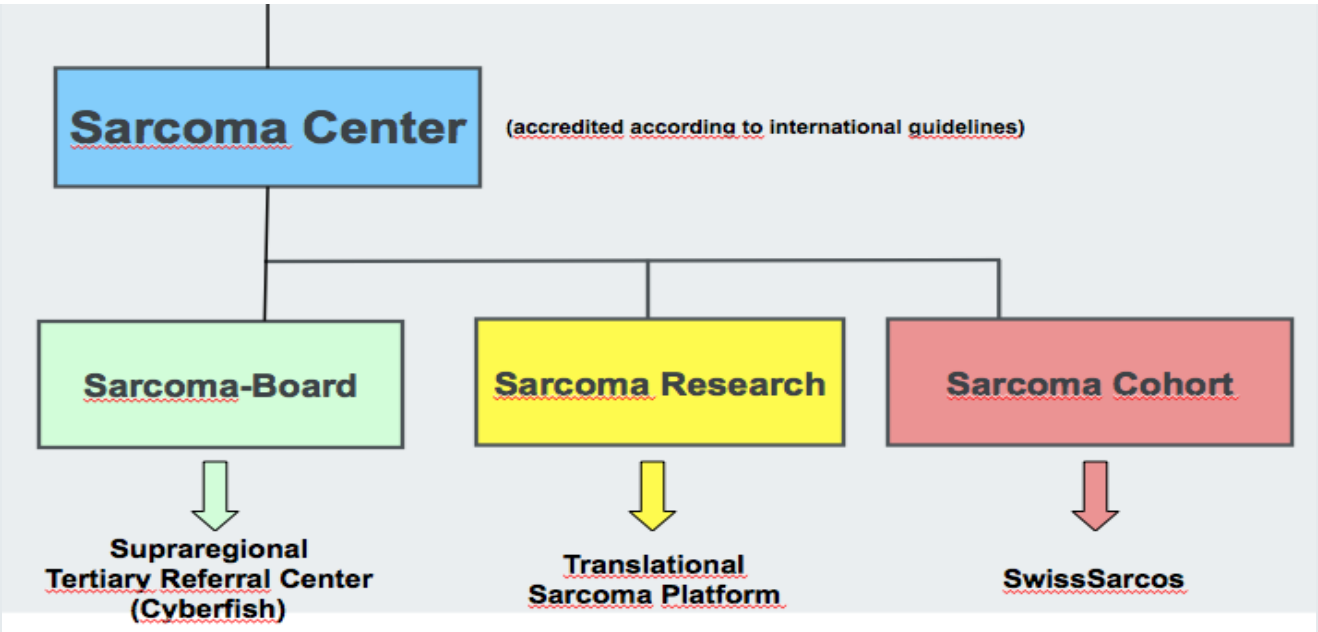
C. **cohort study** SWISSSARCOS

D. **research – tissue collection**

www.sarcoma.ch

SNSAB

Swiss National Sarcoma Advisory Board



SNSAB GUIDELINES

in Anlehnung an ESMO, NCCN, etc

1. **Minimal Work-up requirements**
2. **Therapy**
3. **Surgery (extremity/retroperitoneal)**
4. **Follow-up**
5. **Rad-Onc**
6. **ILP**

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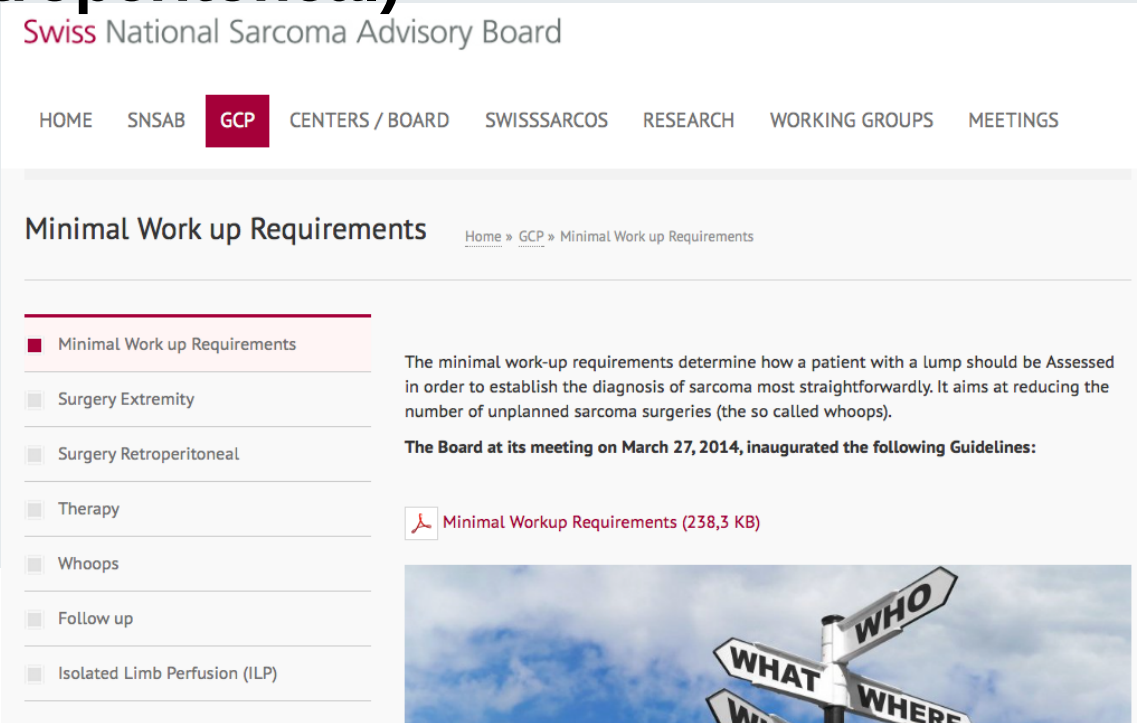
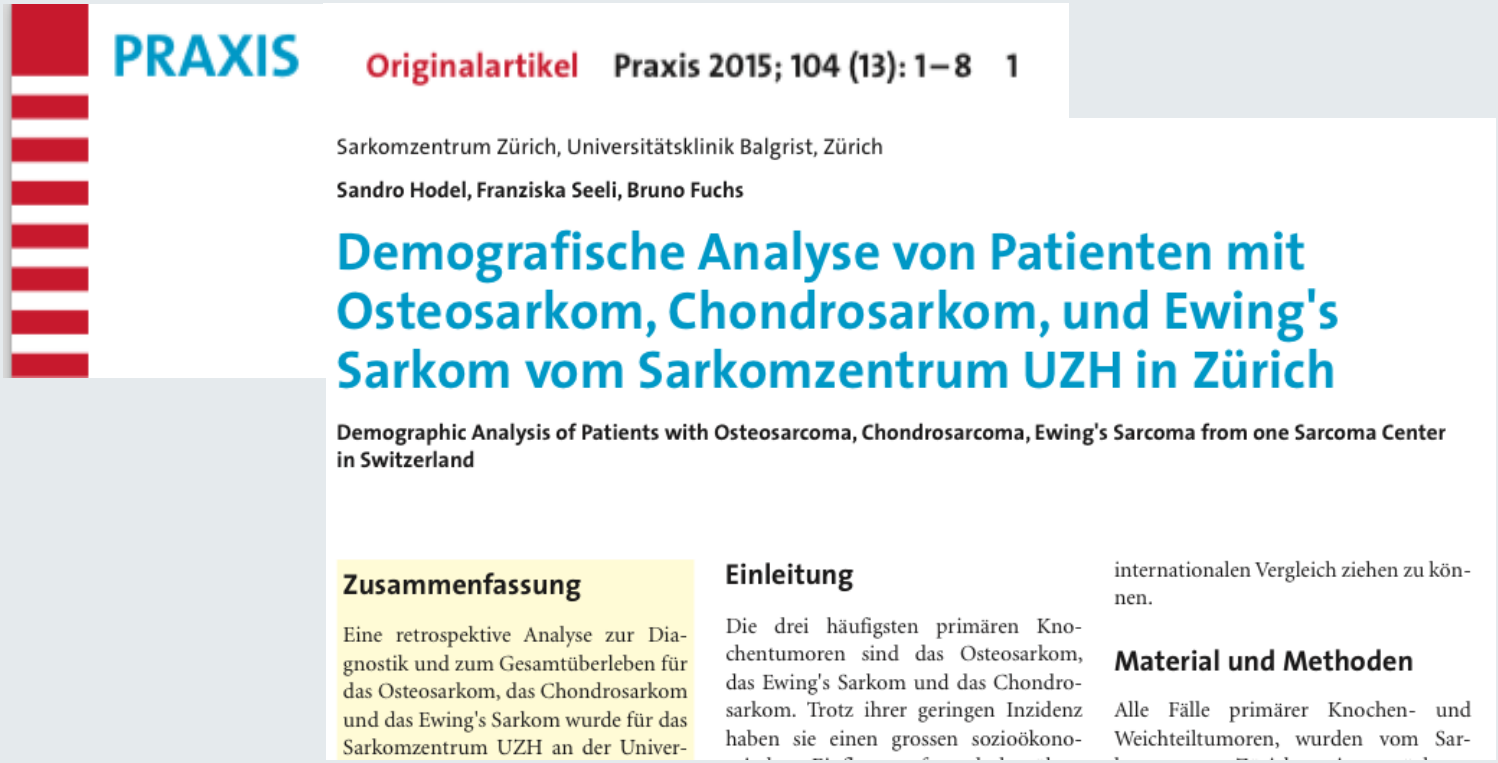


POTENTIAL OF CLINICAL DATA

Sarkomzentrum ZÜRICH



PRAXIS

Originalartikel Praxis 2015; 104 (13): 1–8 1

Sarkomzentrum Zürich, Universitätsklinik Balgrist, Zürich

Sandro Hodel, Franziska Seeli, Bruno Fuchs

Demografische Analyse von Patienten mit Osteosarkom, Chondrosarkom, und Ewing's Sarkom vom Sarkomzentrum UZH in Zürich

Demographic Analysis of Patients with Osteosarcoma, Chondrosarcoma, Ewing's Sarcoma from one Sarcoma Center in Switzerland

Zusammenfassung

Eine retrospektive Analyse zur Diagnostik und zum Gesamtüberleben für das Osteosarkom, das Chondrosarkom und das Ewing's Sarkom wurde für das Sarkomzentrum UZH an der Univer-

Einleitung

Die drei häufigsten primären Knochentumoren sind das Osteosarkom, das Ewing's Sarkom und das Chondrosarkom. Trotz ihrer geringen Inzidenz haben sie einen grossen sozioökono-

internationalen Vergleich ziehen zu können.

Material und Methoden

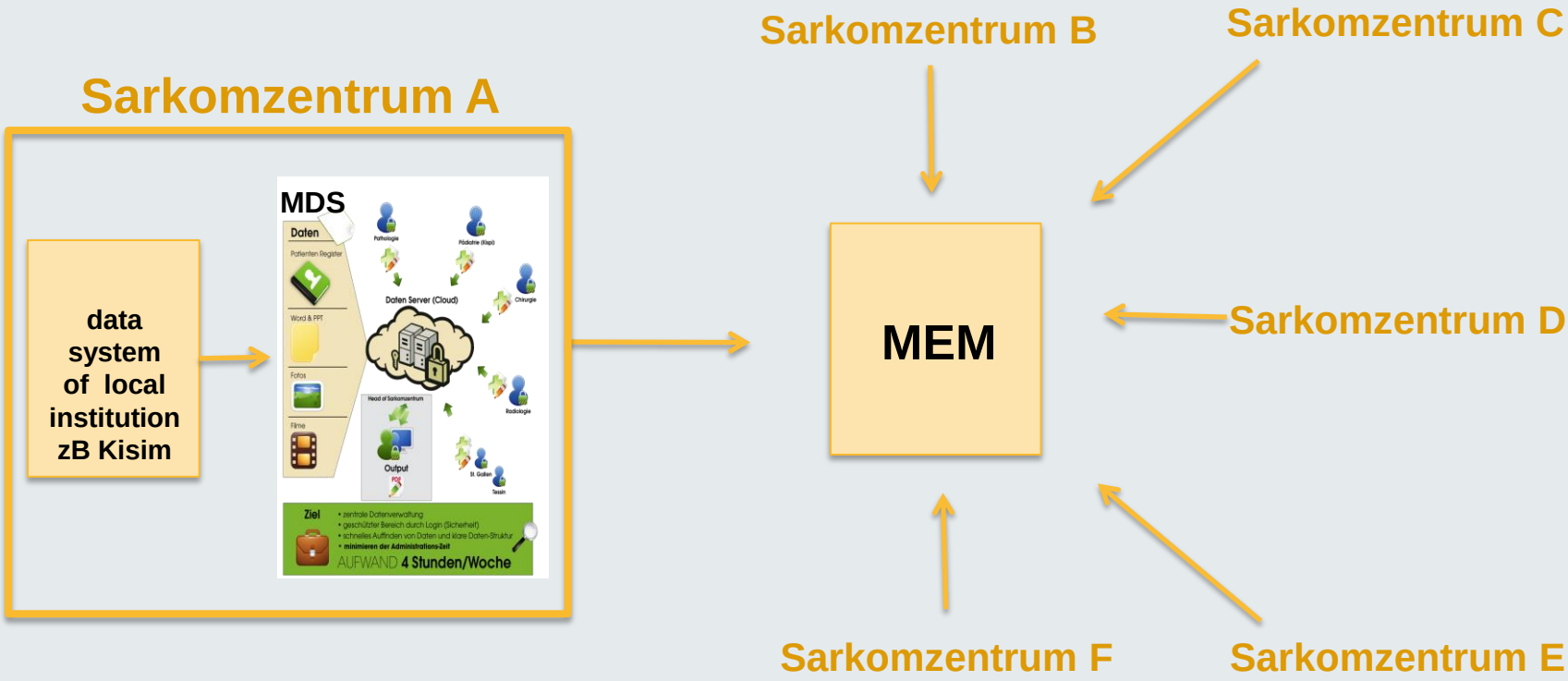
Alle Fälle primärer Knochen- und Weichteiltumoren, wurden vom Sar-

> 200 patients from one single center

COHORT STUDY

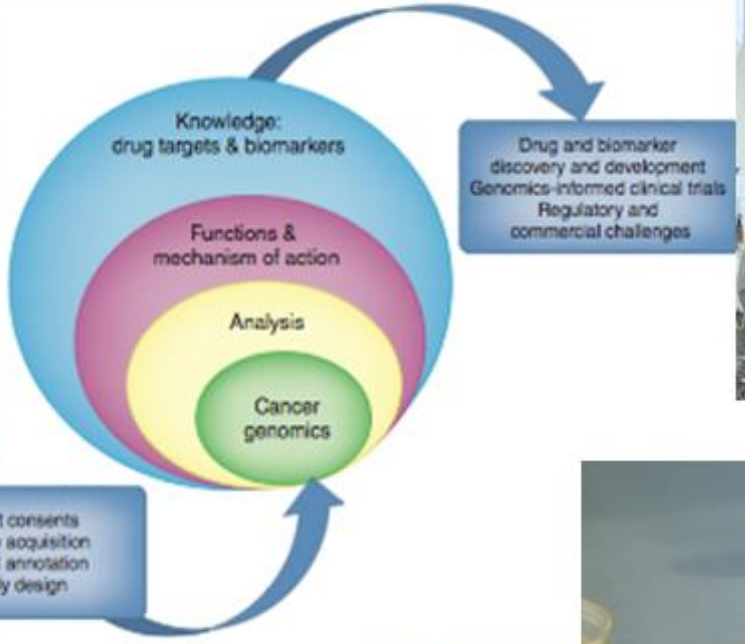
SWISSSARCOS

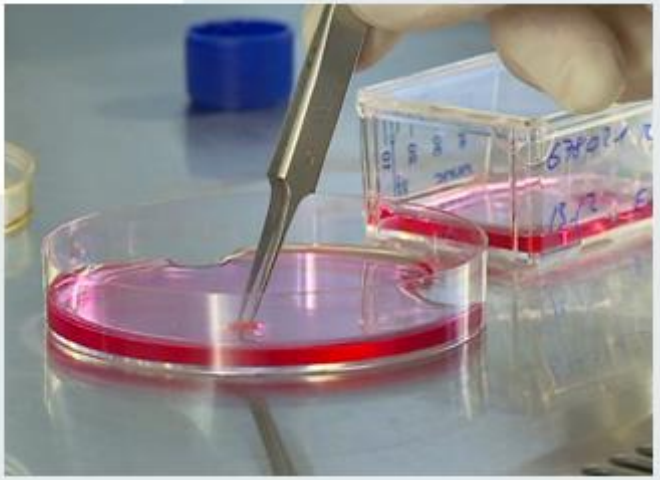
Data Management



ethic approval obtained !

TISSUE COLLECTION

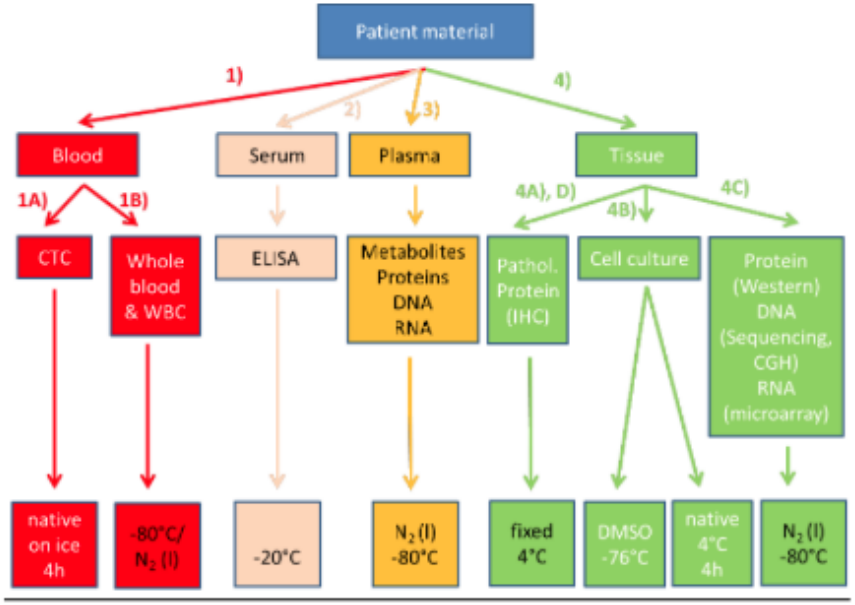


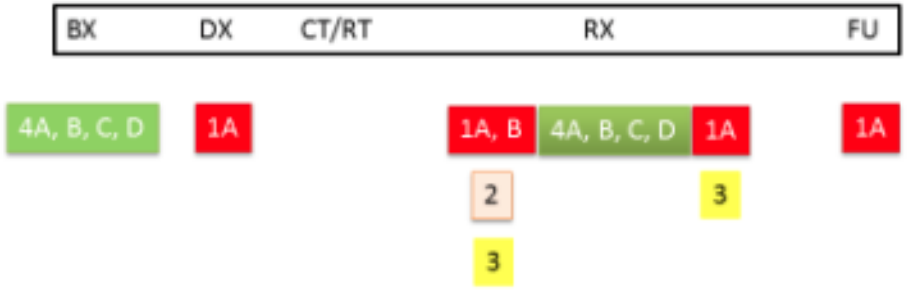


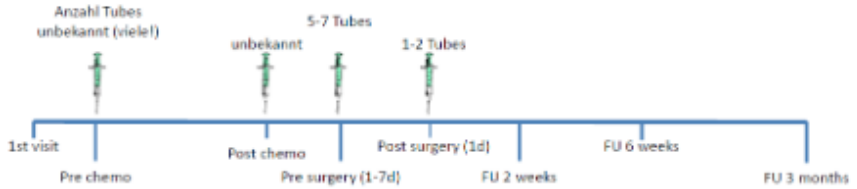
TISSUE COLLECTION

Working Group

What to collect







= medically indicated blood collection

Open questions

Priority for tissue (4A, B, C, D)?

1B) not performed yet (to be dicussed)

4D) not performed yet (to be discussed)

Decentralized storage, centralized registration in satabase?

Centralized database read only?

Who updates registration?

Who decides which samples can be used for studies?

Anonymization (no PID)?

www.sarcoma.ch

Zürich

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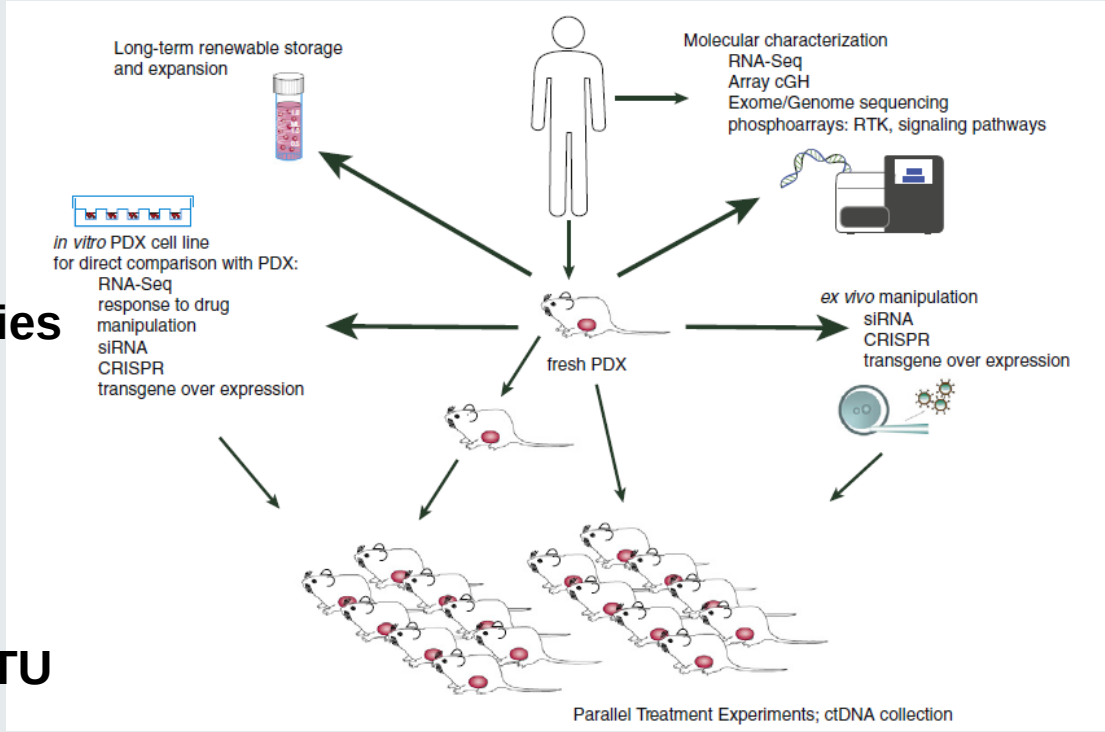
IN VITRO & IN VIVO MODELLING

Advantages of:

- established cell lines:
  - ease of propagation
  - mechanistic/functional studies

- 1° tumor cells

  biological relevance ⬆
  mimicks closely patient‘s TU

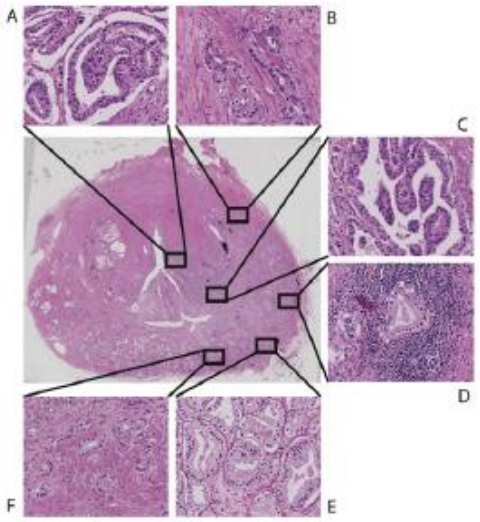


IN VITRO & IN VIVO MODELLING

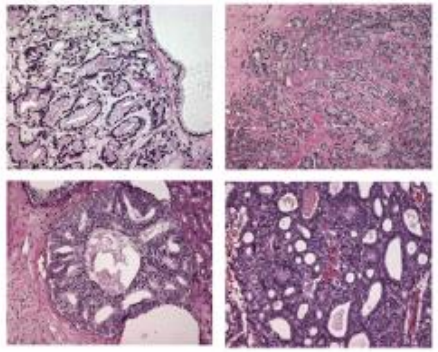
heterogenous

homogenous

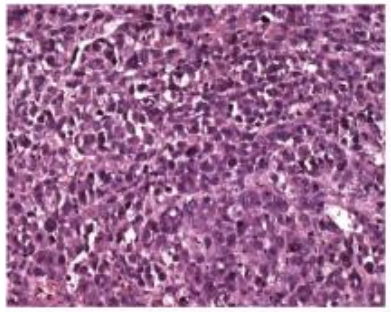
patient's tumor



PDX



cell line xenograft

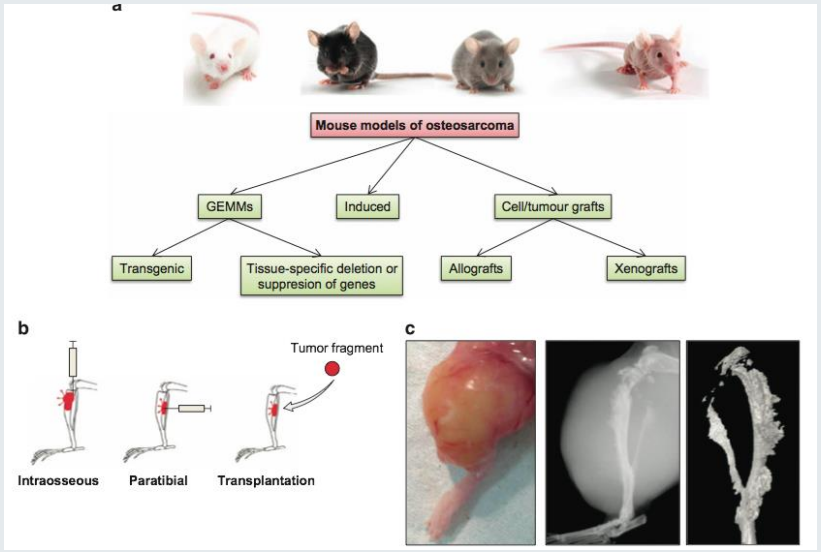


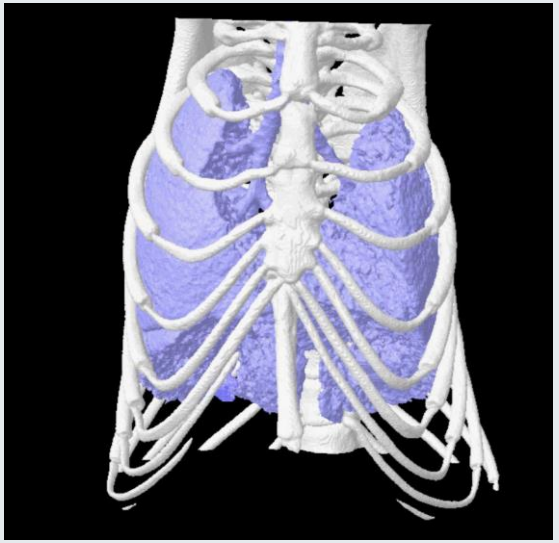
cell line in vitro

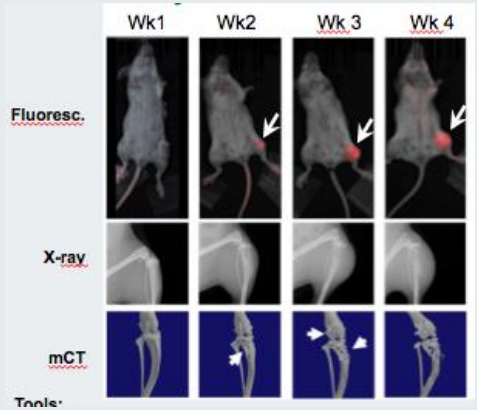
Choi et al Advanced Drug Del Reviews 79-80:222-37, 2014

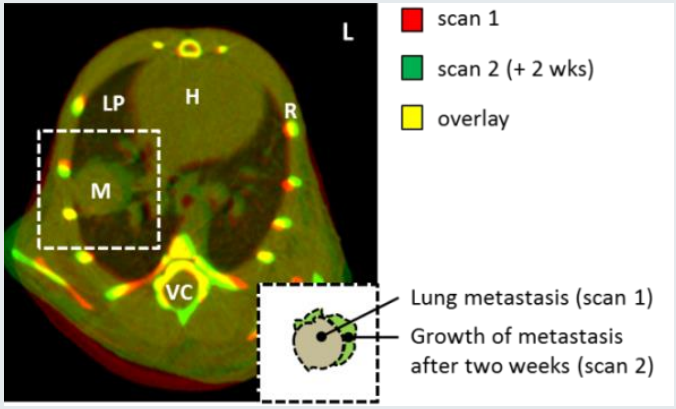
MOUSE MODELLING OF OS

Orthotopic Sarcoma Mouse Models







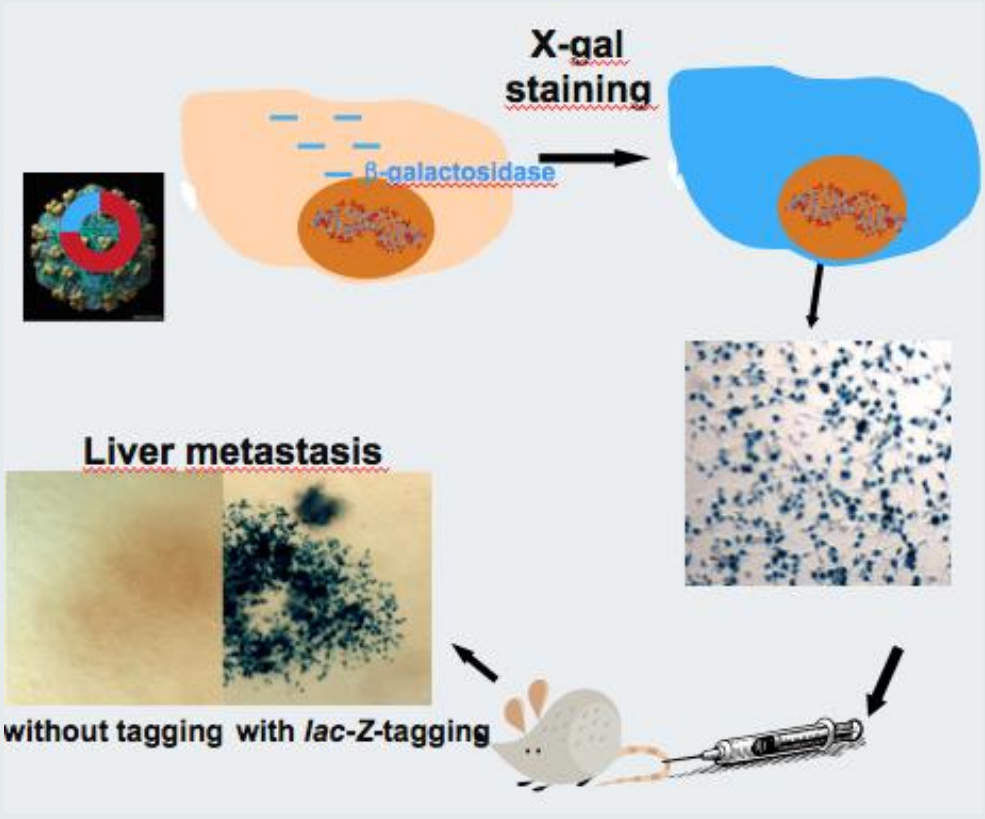


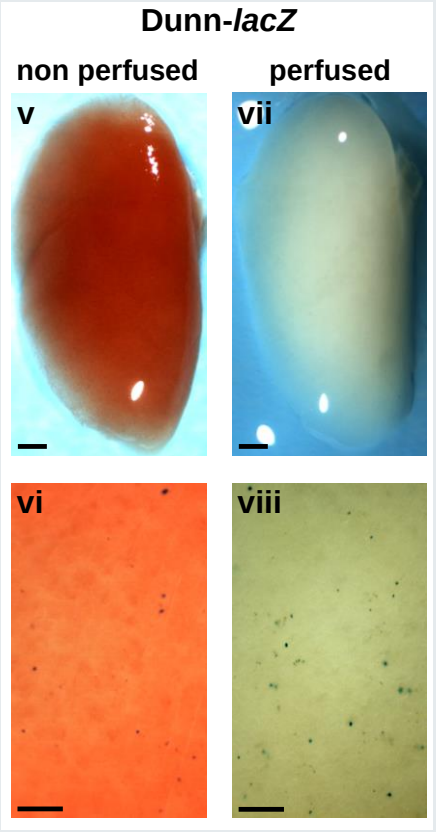
Botter S et al, in „Bone Cancer“;2:349-63, 2015 (2nd edition)

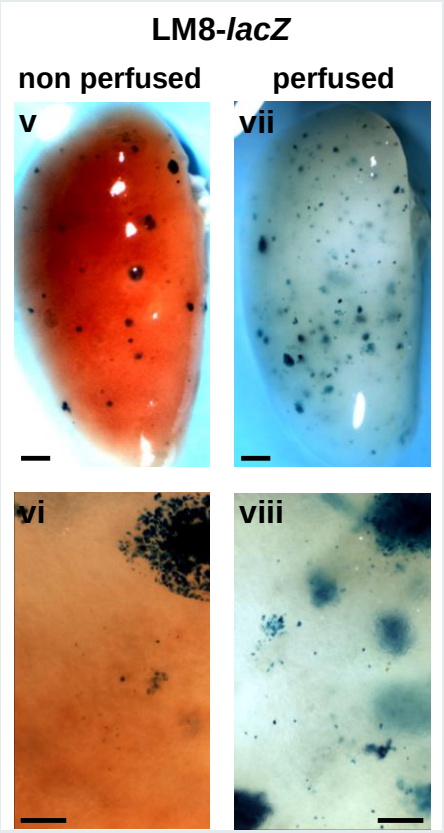
Universität Zürich[UZH]

Sarkomzentrum ZÜRICH

MOUSE MODELLING OF OS
LacZ tagging of individual cells





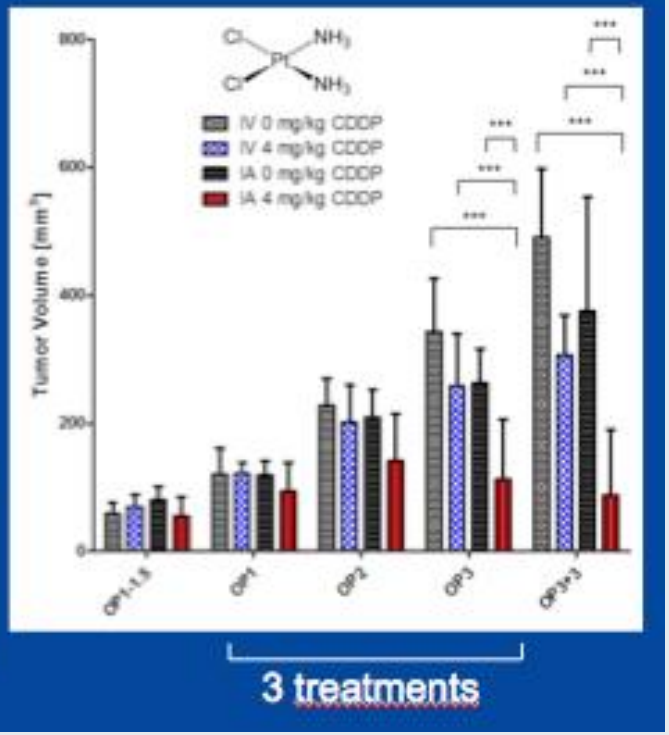


Arlt M et al. J Orthop Res 29:938-46, 2011

MOUSE MODELLING OF OS
Intraarterial Drug Administration

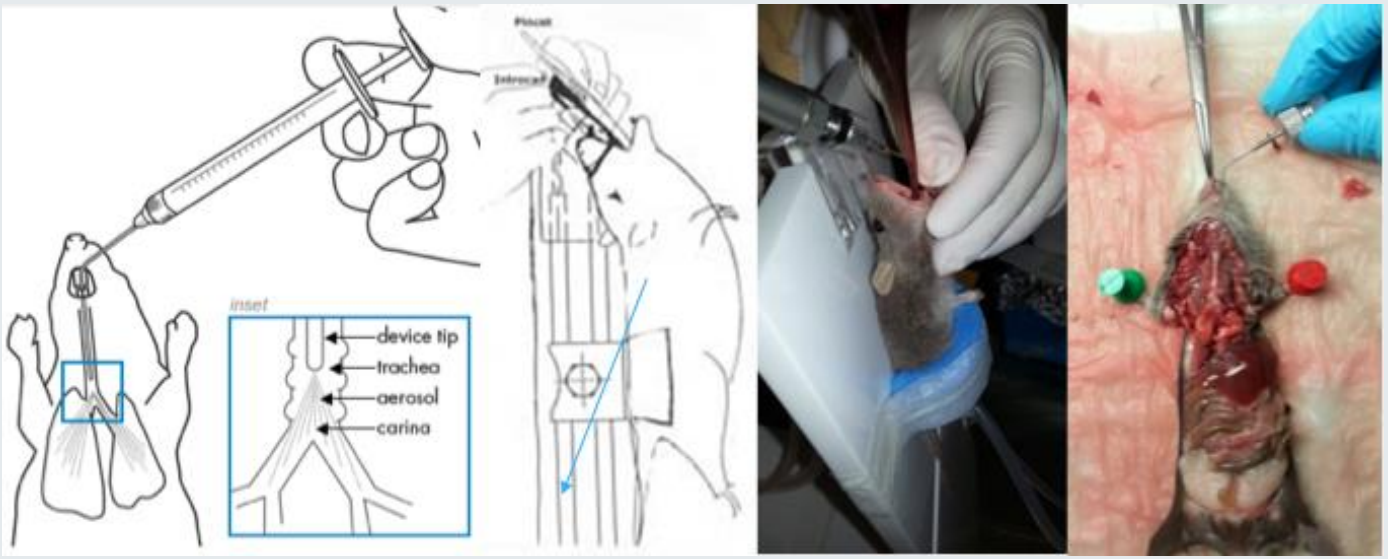
Cisplatin: systemic vs. local infusion

Infusion platform for other drug classes



Robl B et al. In preparation

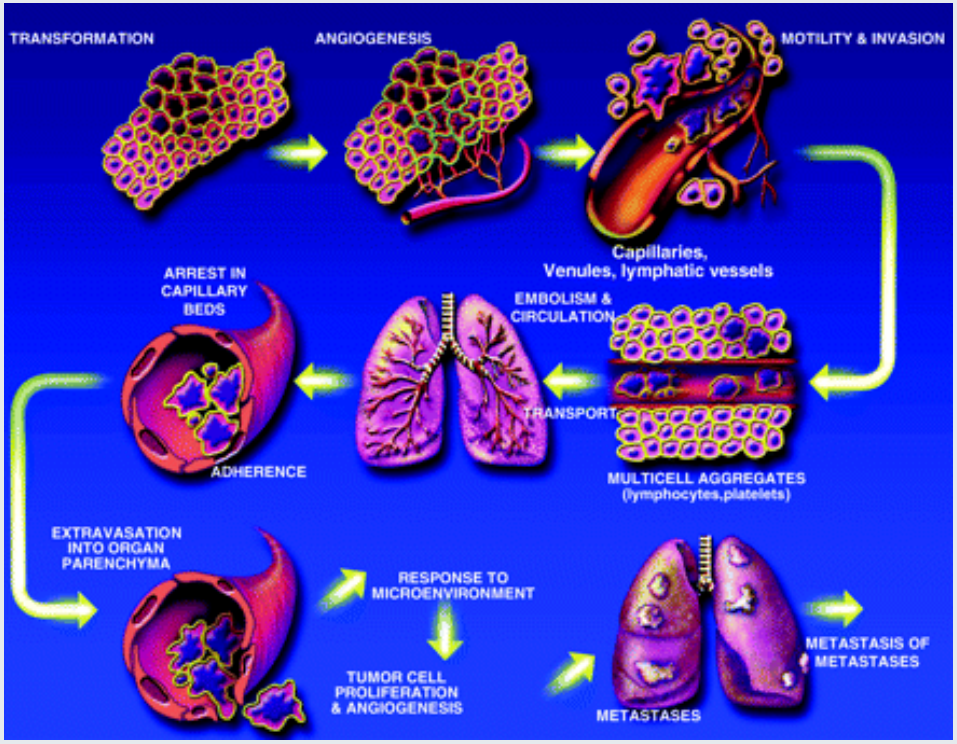
MOUSE MODELLING OF OS
Intratracheal Drug Administration

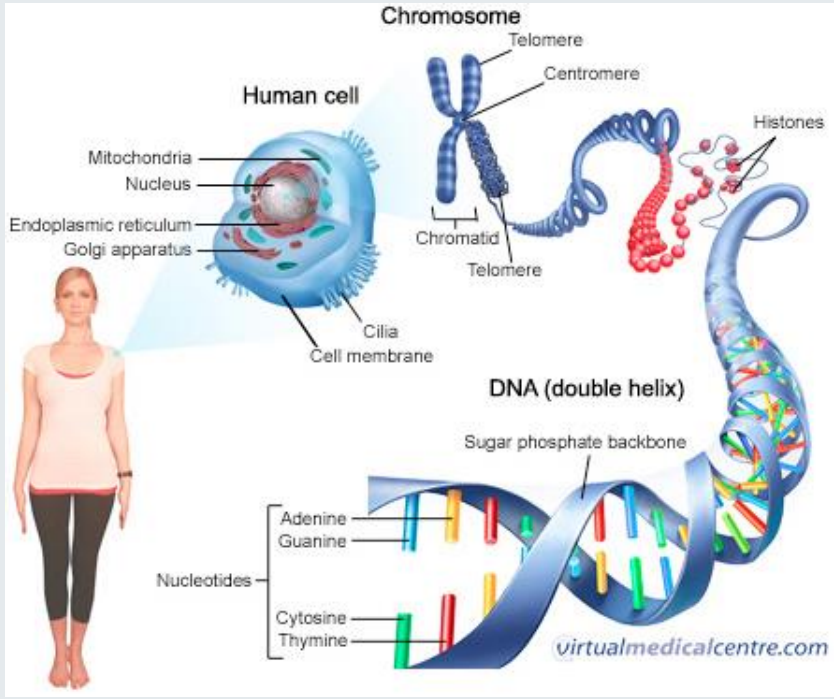


Neklyodova O et al. In preparation

PERSONALIZED MEDICINE

Where do we focus on ?





→ several areas of interest !

PERSONALIZED MEDICINE
1. Patient Stratification

Classification of Bone and Soft Tissue Sarcomas WHO 2013

ADIPOCYTIC TUMORS

benign
2.1.1. lipoma (angio-/spindle-/myo-/chondroid/lipoblastoma)
2.1.2. hibernoma
intermediate (locally aggressive)
2.2.1. atypic lipomatous tumor
2.2.2 well differentiated liposarcoma (retroperitoneal)
malignant
2.4.1. dedifferentiated liposarcoma
2.4.2. myxoid liposarcoma (incl round cell)
2.4.3. pleomorphic liposarcoma
2.4.4. liposarcoma not otherwise specified

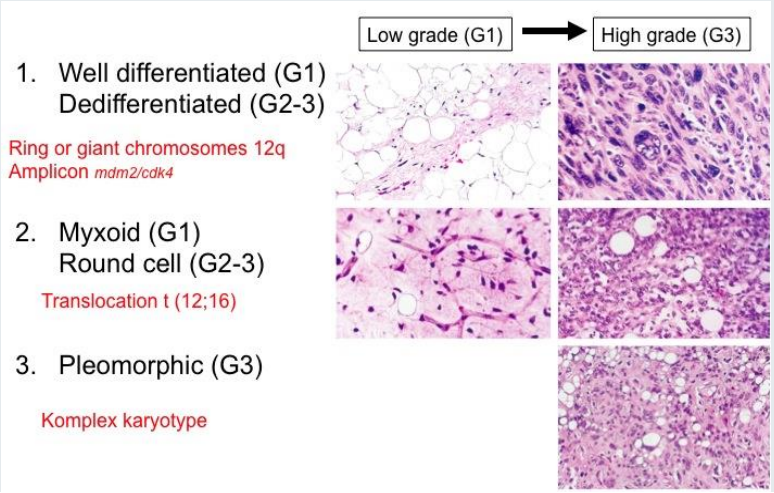


Table 2
Prognosis depending on LPS subtype

Subtype	Recurrence, %	Metastasis, %	OS, %	DSS, %	Prognosis Factors
WDLPS	13–46 (extremity) 91 (retroperitoneal)	Very low	76–93	86	Location; margin
DDLPS	18–57	13–47	54–64	66–89	Location; mitotic count
MLPS/RCLPS	7–28	10–58	40–75	69–100	Age; RC-component
PLPS	16–45	32–44	0–63	50	Mostly none

Table 1
Overview of LPS subtypes

Subtype	Pathology	Molecular Characteristics/ "Actionable" Targets	MRI/CT Appearance
ALT/WDLPS	Low grade, positive IHC for MDM2, CDK4, p16	MDM2 and CDK4 amplifications	Large encapsulated lipomatous mass (high signal intensity both in T1-weighted and T2-weighted MRI) with thick internal septations; Signal loss on fat-saturated T1-weighted images, and focal nodules (>1 cm is suggestive of a DDLPS)
DDLPS	High grade, positive IHC for MDM2, CDK4, p16	MDM2 and CDK4 amplifications	
MLPS and RCLPS	Low grade (percentage of round cells important for grading)	*FUS-CHOP* fusion gene, PI3K mutations (~20%)	Pathognomonically low signal intensity in T1-weighted and marked signal intensity in T2-weighted MRI
PLPS	High grade, pleomorphic, cellular sarcoma	Complex structural rearrangements	Nonspecific soft tissue mass, often including areas of necrosis and hemorrhage

miRNAs as discriminators in liposarcoma

PERSONALIZED MEDICINE

1. Patient Stratification

PID	Sex	Age at Dx	Diagnosis	Grade I II III	Location	Total Dosage, Gy	Date of surgery	TU volume in cc	Balgrist Resection (R0=1, R1=2, R2=3)	last FU	state of disease	Death	local Recurrence	Metastasis	Meta. Loc.	Mutation	Round Cells (Y/N)	MDM2
746461	m	42	PMLS	3	Lower Extremity	50	26.08.2014	1368	1	28.12.2014	NED	n	n	n			n	n
732538	m	51	MLS	3	Lower Extremity	50	03.12.2013	1802	1	03.07.2014	NED	n	n	n		FUS/DDIT3 Typ 3	y	
712827	f	37	MLS	3	Lower Extremity	50	09.10.2012	262	2	26.09.2014	NED	n	n	n		FUS/DDIT3 Typ 5	y	
706598	m	45	MLS	1	Lower Extremity	50	28.05.2013	936	1	28.08.2014	NED	n	n	y	Lower Extremity	x	y	
714162	m	78	MLS	x	Upper Extremity	50	13.11.2012	700	1	05.06.2014	NED	n	n	n		FUS/DDIT3 Typ 2	n	
304940	m	50	MLS	1	Lower Extremity	50	21.01.2014	432	1	10.07.2014	NED	n	n	n		x	n	
739502	m	18	MLS	1	Lower Extremity	50	01.04.2014	64	1	30.07.2014	NED	n	n	n		DDIT3/CHOP	n	
738656	m	44	MLS	1	Lower Extremity	50	15.04.2014	1540	1	03.09.2014	NED	n	n	n		FUS/DDIT3 Typ 3	n	
590667	f	39	MLS	1	Thorax	50	29.04.2014	682	1	23.07.2014	AWD	n	n	y	Gluteal	x	n	y
749209	f	35	MLS	1	Lower Extremity	50	09.07.2014	294	1	29.10.2014	NED	n	n	n		FUS/DDIT3 Typ 2	n	
661216	m	41	MLS	x	Lower Extremity	50	01.07.2014	1141	1	16.10.2014	NED	n	n	n		FUS/DDIT3 Typ 2	n	
746689	m	49	MLS	x	Upper Extremity	50	16.09.2014	267	1	17.12.2014	AWD	n	n	y	Upper Extremity	FUS/DDIT3 Typ 2	n	
734366	m	54	MLS	3	Lower Extremity	50	07.01.2014	441	1	03.07.2014	NED	n	n	n		x	y	
749339	f	52	MLS	x	Lower Extremity	50	28.10.2014	72	1	12.11.2014	NED	n	n	n		FUS/DDIT3 Typ	n	
625162	m	78	DDLS	2	Lower Extremity	50	30.09.2014	54	1	24.12.2014	AWD	n	n	y	Lungs, Spleen, Bone	x	n	y
723755	m	67	DDLS	2	Lower Extremity	50	28.05.2013	1472	1	27.08.2014	NED	n	n	n		x	n	y
680808																		
669696																		
751212																		
662727																		
734417	m	75	ALT	1	Lower Extremity	50	04.03.2014	1805	1	04.06.2014	NED	n	n	n		x	n	y
518731	f	46	ALT	1	Upper Extremity	50	03.12.2013	300	1	13.03.2014	NED	n	n	n		x	n	y
705359	f	59	ALT	1	Upper Extremity	50	17.04.2012	84	1	11.09.2013	NED	n	n	n		x	n	y
714975	m	71	ALT	1	Lower Extremity	50	04.12.2012	6.75	1	12.03.2014	NED	n	n	n		x	n	y
512208	m	57	ALT	1	Lower Extremity	50	29.01.2013	2208	1	23.10.2014	NED	n	n	n		x	n	y
735839	f	50	ALT	1	Lower Extremity	50	01.04.2014	1332	1	09.07.2014	NED	n	n	n		x		x
711964	m	63	lipoma		Upper Extremity		10.07.2012	540		10.07.2012	NED	n	n	n				
706886	f	53	lipoma		Upper Extremity		27.03.2012	60		11.04.2012	NED	n	n	n				
708924	m	43	lipoma		Thorax		29.05.2012	80		29.08.2012	NED	n	n	n				
706379	f	57	lipoma		Lower Extremity		12.06.2012	12		01.08.2012	NED	n	n	n				
711209	f	62	lipoma		Upper Extremity		30.10.2012	28		27.03.2013	NED	n	n	n				
710325	m	44	lipoma		Lower Extremity		27.11.2012	7.5		08.11.2012	NED	n	n	n				
675394	f	45	lipoma		Lower Extremity		09.10.2012	24		24.10.2012	NED	n	n	n				
709604	m	63	lipoma		Lower Extremity		23.10.2012	308		07.11.2012	NED	n	n	n				
711151	f	33	lipoma		Back/Thorax		18.09.2012	40.5		04.10.2012	NED	n	n	n				
719055	f	52	lipoma		Upper Extremity		18.12.2012	382		13.03.2013	NED	n	n	n				

36 patients in total; MLS: 13; L: 10; ALT: 7; DDLS: 5; PML: 1

miRNAs as discriminators in liposarcoma

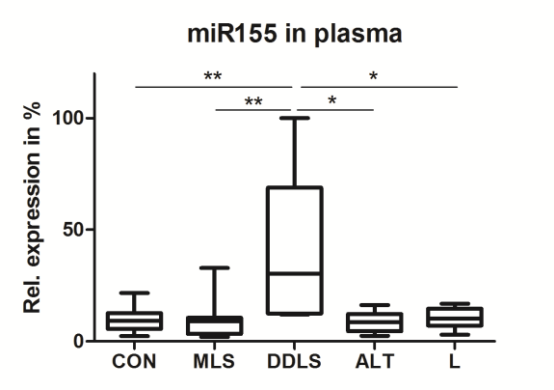
Universität Zürich[UZH]

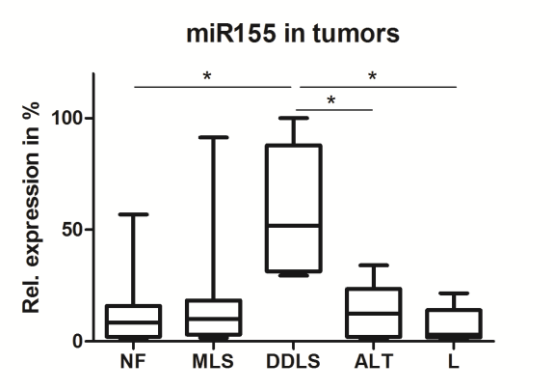
Sarkomzentrum ZÜRICH

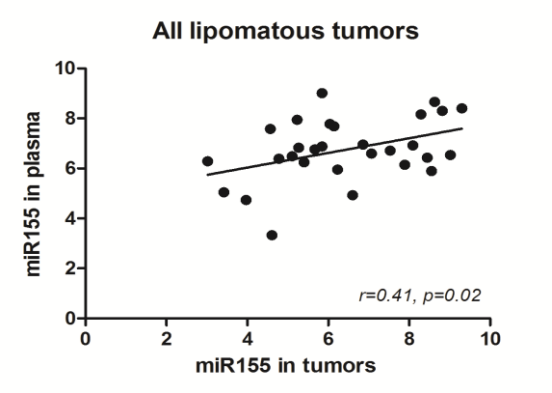
PERSONALIZED MEDICINE

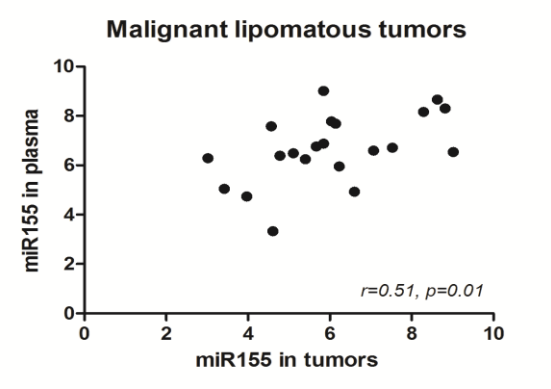
1. Patient Stratification

correlation of miR155 expression in plasma & tumor samples





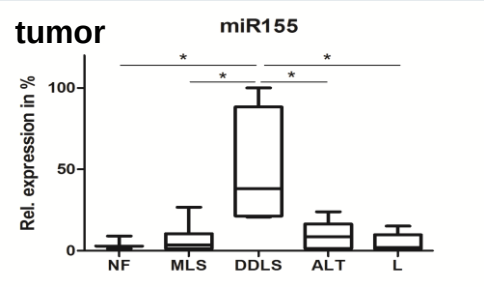


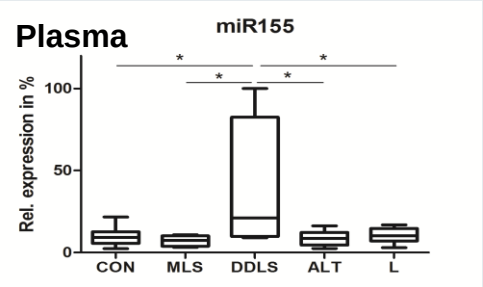


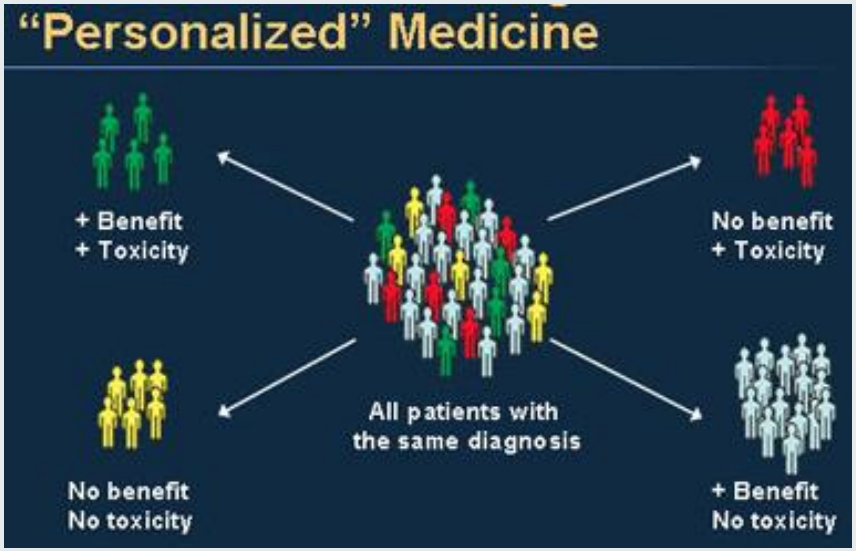
miRNAs as discriminators in liposarcoma

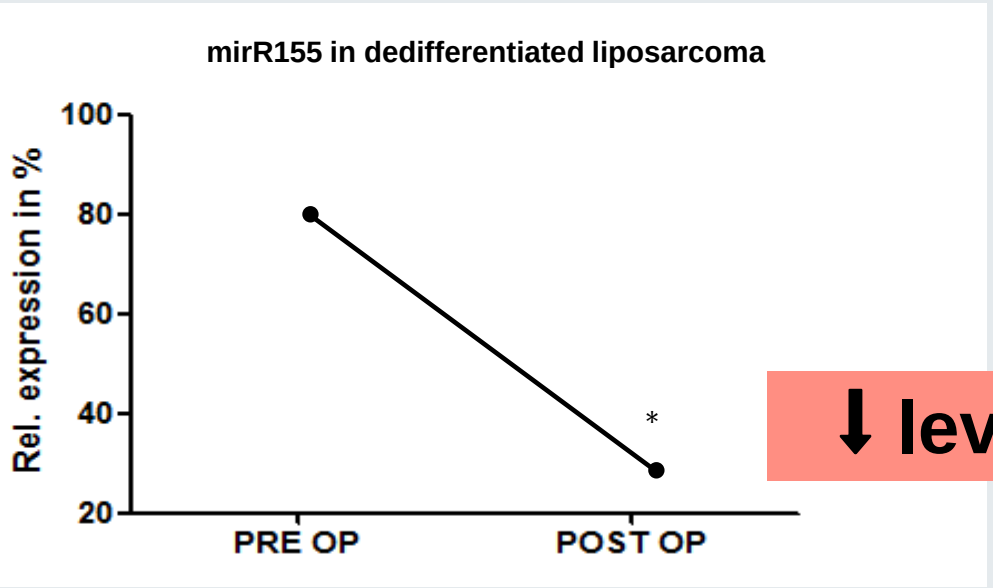
PERSONALIZED MEDICINE

1. Patient Stratification









⬇ levels 24h post surgery !

miRNAs as discriminators in liposarcoma

PERSONALIZED MEDICINE
2. in-vitro 3D & in-vivo modelling

Contents lists available at ScienceDirect

Journal of Biotechnology

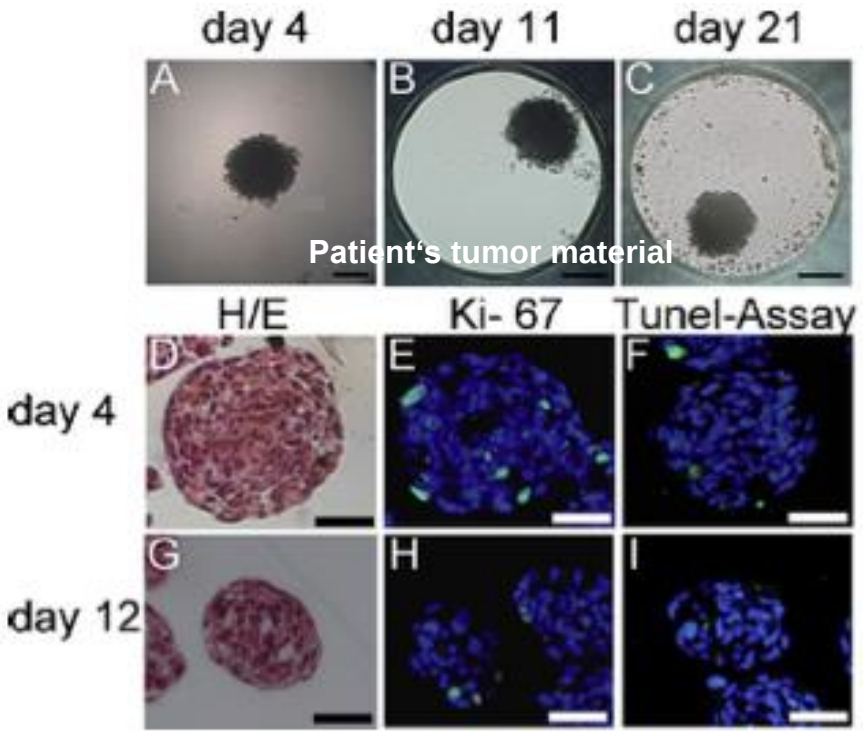
journal homepage: www.elsevier.com/locate/jbiotec

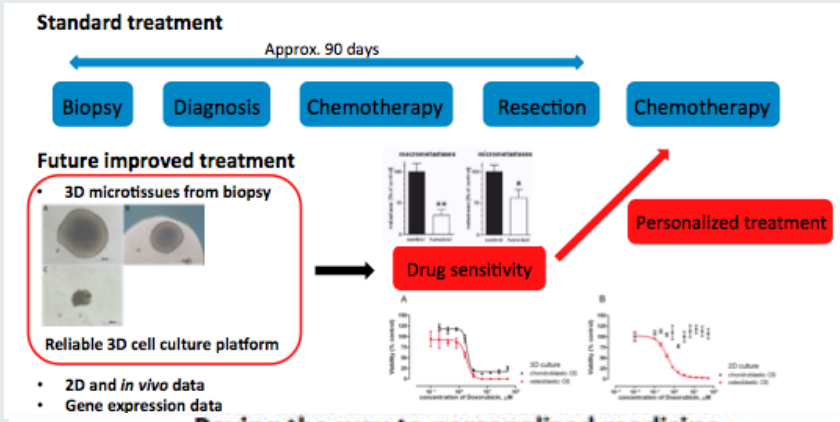
An *in vitro* osteosarcoma 3D microtissue model for drug development

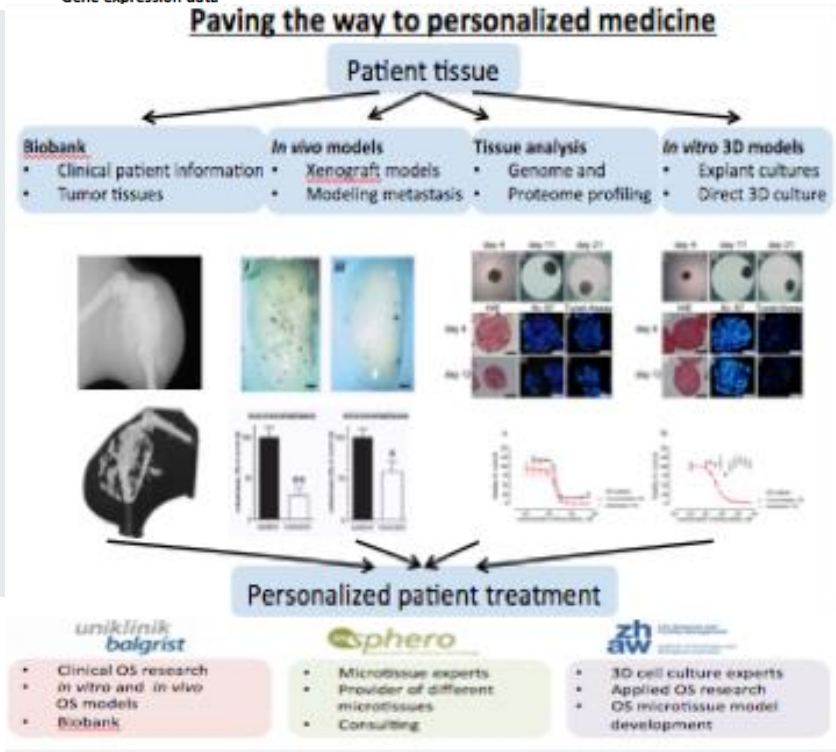
Markus Rimann[a,1], Sandra Laternser[a,1], Ana Gvozdenovic[b], Roman Muff[b], Bruno Fuchs[b], Jens M. Kelm[c], Ursula Graf-Hausner[a,*]

[a] Institute of Chemistry and Biological Chemistry (ICBC), Zurich University of Applied Sciences, Waedenswil, Switzerland
[b] Laboratory for Orthopedic Research, Department of Orthopedics, University of Zurich, Switzerland
[c] InSphero AG, Schlieren, Switzerland

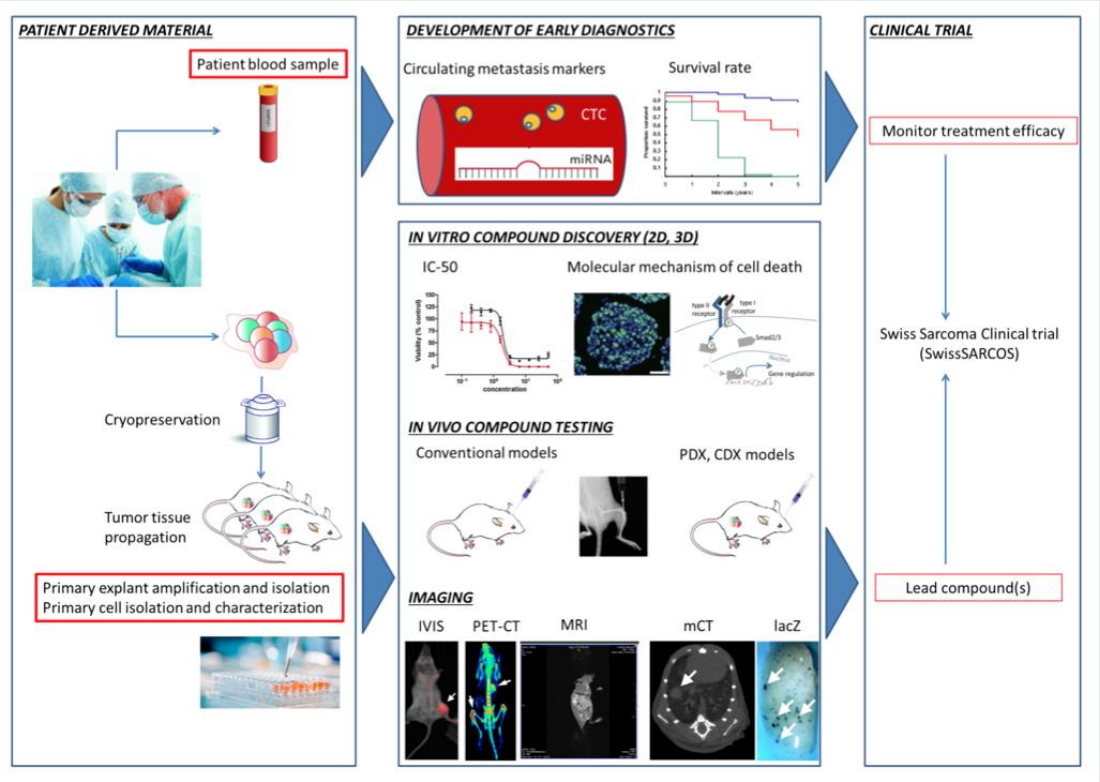
Journal of Biotechnology 189 (2014) 129–135







PERSONALIZED MEDICINE
2. in-vitro 3D & in-vivo modelling



NFP - application 2017 – 2020:

3D-Tissue Models –
new perspectives for medicine

→personalized therapy in the future !

PERSONALIZED MEDICINE

3. Dormancy

Research Article

Reduced Latency in the Metastatic Niche Contributes to the More Aggressive Phenotype of LM8 Compared to Dunn Osteosarcoma Cells

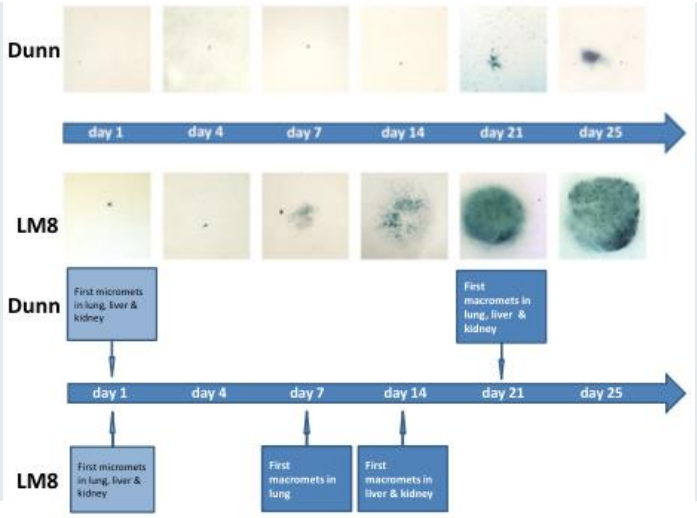
Matthias J. E. Arlt, Ingo J. Banke, Josefine Bertz, Ram Mohan Ram Kumar, Roman Muff, Walter Born, and Bruno Fuchs

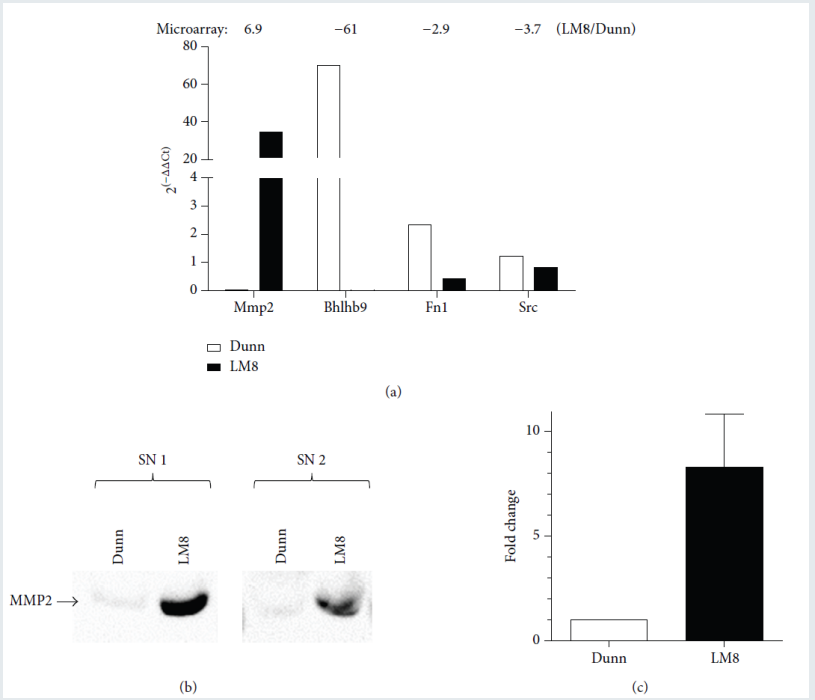
Laboratory for Orthopedic Research, Department of Orthopedics, Balgrist University Hospital, University of Zurich, Forchstrasse 340, 8008 Zurich, Switzerland

Correspondence should be addressed to Matthias J. E. Arlt; marlt@research.balgrist.ch

Received 24 July 2013; Revised 13 October 2013; Accepted 13 October 2013

	Cell Lines		Subcutaneous Tumors		Intratibial Tumors	
Genes down-regulated in LM8	**symbol**	**fold change**	**p value**	**fold change**	**p value**	**fold change**	**p value**
basic helix-loop-helix domain containing, class B9	Bhlhb9	-60.98	8.46E-13	-24.93	9.47E-10	-15.67	2.45E-04
fibronectin 1	Fn1	-2.95	5.69E-08	0.9001	0.4485	0.9257	0.9272
transforming growth factor, beta 2	Tgfb2	-2.43	6.09E-06	-2.38	4.93E-05	-4.07	8.84E-03
Rous sarcoma oncogene	Src	-2.36	2.24E-06	-2.70	0.0001217	0.8423	0.759
tropomyosin 1, alpha	Tpm1	-2.28	2.53E-07	-2.23	5.30E-05	0.6487	0.358625
transforming growth factor, beta receptor I	Tgfbr1	-2.15	5.06E-06	1.15085	0.2542225	0.773225	3.76E-01
Genes up-regulated in LM8							
connective tissue growth factor	Ctgf	2.72	2.18E-04	0.5983	0.0307	0.8579	0.858





Arlt M et al, Sarcoma 2013 ID 404962

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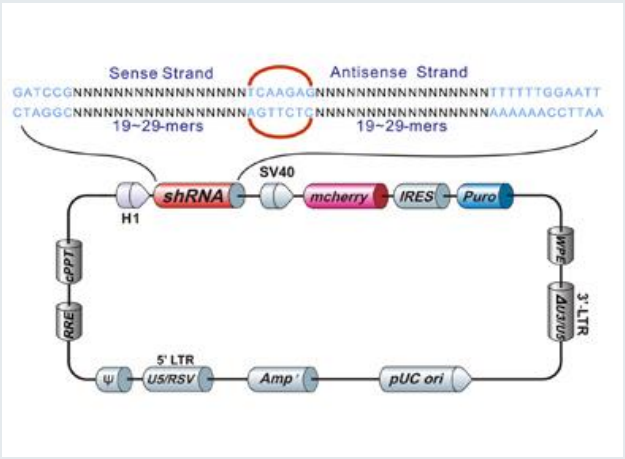
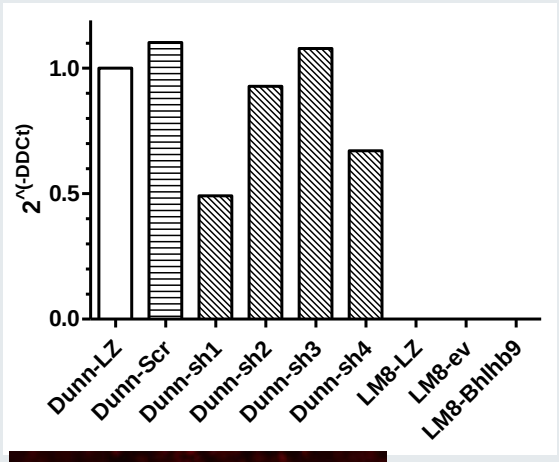
PERSONALIZED MEDICINE

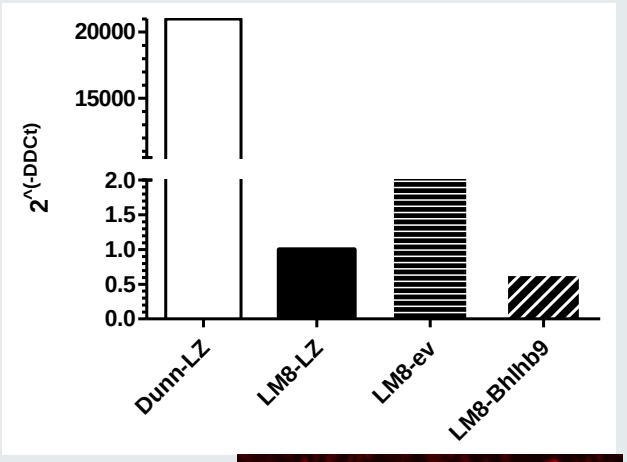
3. Dormancy

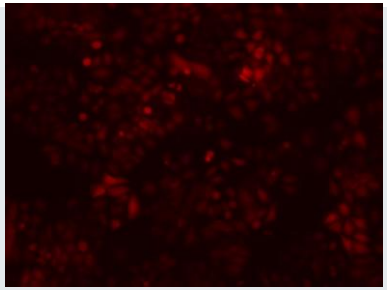
Lenti Vector for MSH043018-1-LVRH1MP



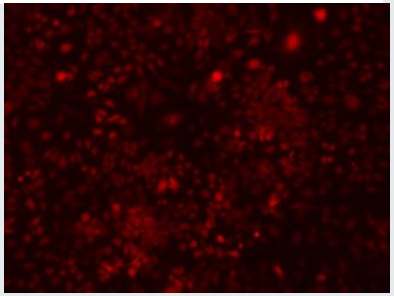
Bhlhb9 overexpression & knockdown







mCherry Co-expression



→ *in vivo experiments underway !*

PERSONALIZED MEDICINE
4. Circulating Tumor Cells

Rationale

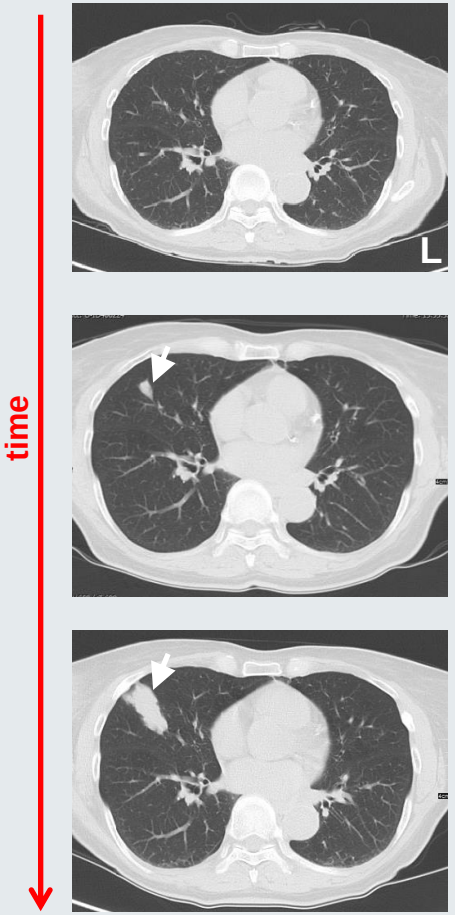
- Metastatic disease: predictor of patient survival
- Early diagnostics guide therapeutic decision making

Markers of metastases in blood circulation

- Circulating (metastatic) tumor cells; CTCs
- Circulating tumor-derived molecules: miRNA, proteins, DNA

Detection tools for CTCs:

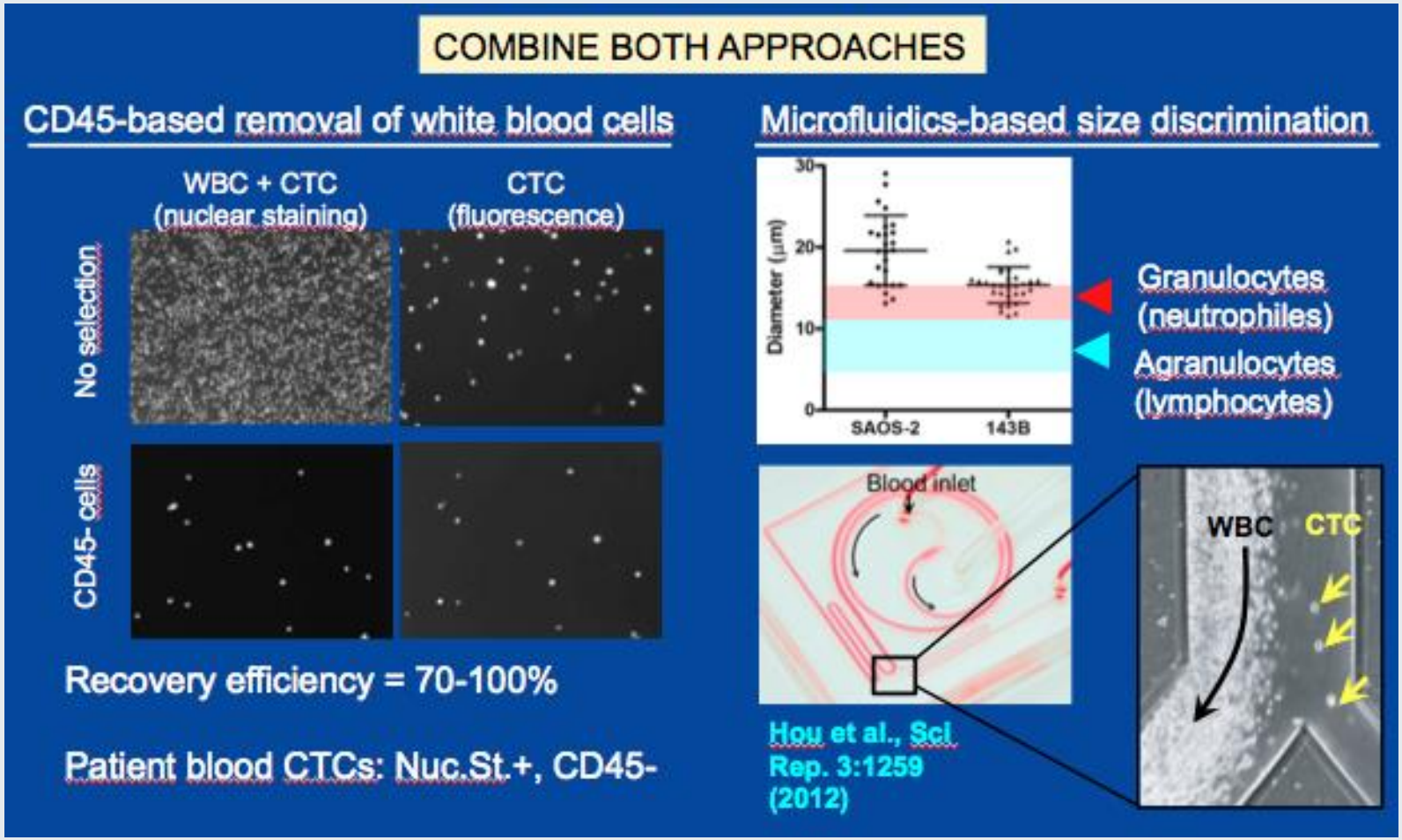
- Metastatic epithelial cancers→ **≥5 CTCs/7.5 ml**
  Cell surface marker-dependent detection
  e.g. Bidard et al., Lancet Oncol. 15(4): 406 (2014)
- Metastatic mesenchymal sarcomas→
  No cell surface markers



How to detect a few CTCs between millions of white blood cells ?

PERSONALIZED MEDICINE

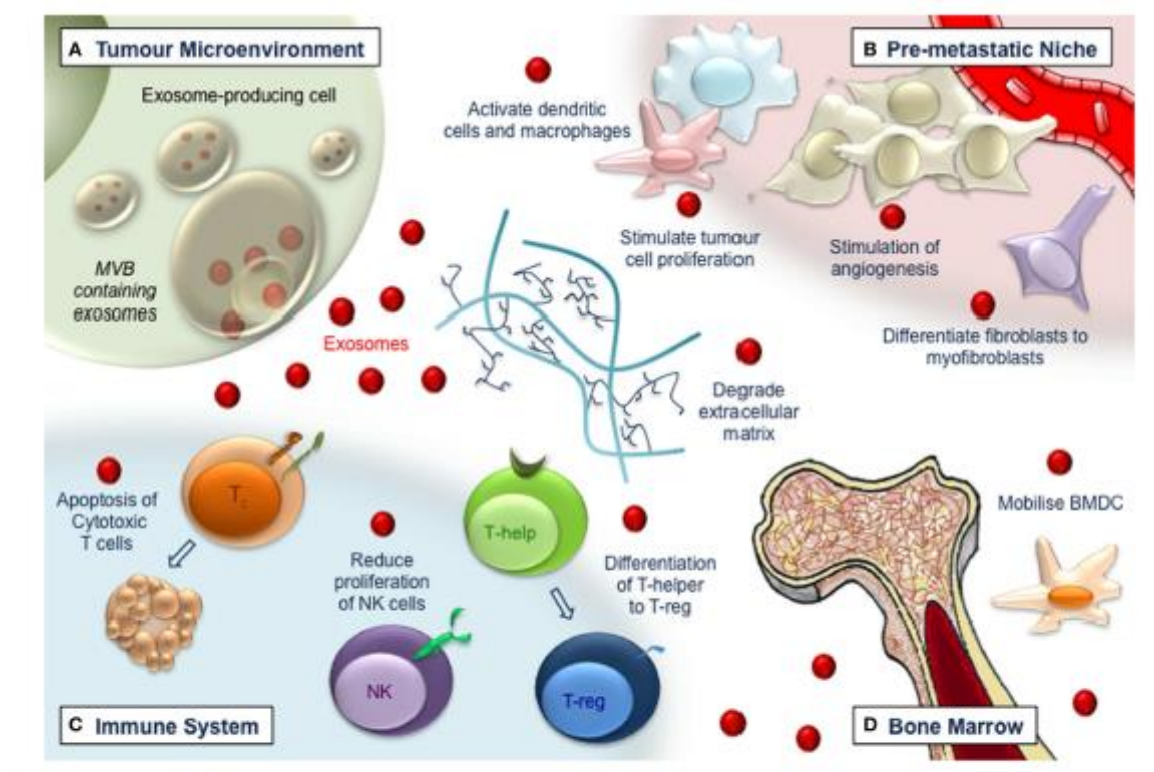
4. Circulating Tumor Cells



→ we have blood available from 400 patients !

PERSONALIZED MEDICINE

5. Exosomes (Tumor Derived Microvesicles)



- **Exosomes are small membrane extracellular vesicles (30-100nm) that mediate local and systemic cell communication through the transfer of mRNA, microRNAs and proteins.**

- **Exosomal mRNA and miRNA differ from the donor cell!**

PERSONALIZED MEDICINE

5. Exosomes (Tumor Derived Microvesicles)

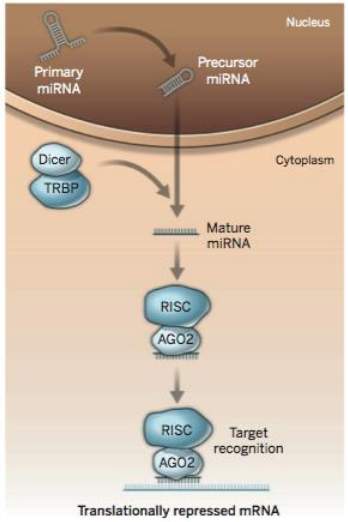
CANCER

Malicious exosomes

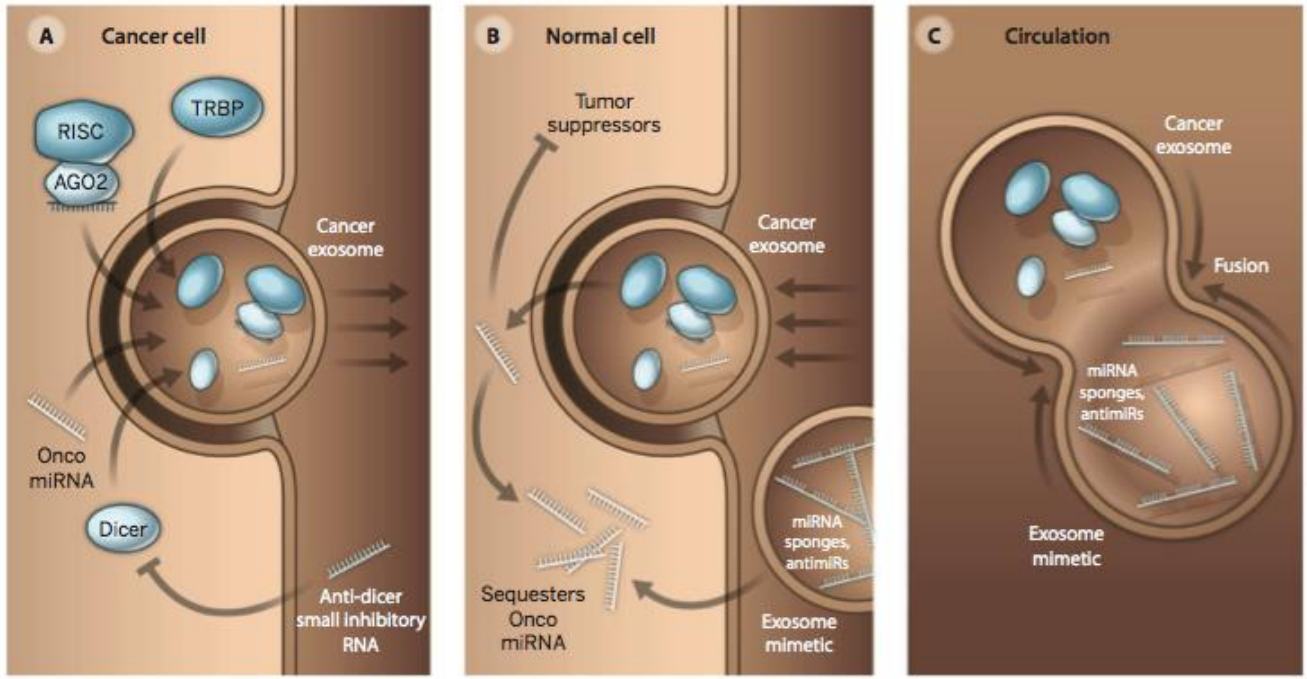
Nanovesicles derived from cells of cancer patients carry microRNAs that initiate tumor growth in normal cells

By **Eleni Anastasiadou** *and* **Frank J. Slack**

Nanovesicles known as exosomes are secreted from a variety of cell types and circulate in biological fluids such as urine and plasma. These exosomes "hijack" membrane components and cytoplasmic contents of these cells and play an important role in intercellular communication, often inducing physiological changes in recipient cells by transferring bioactive lipids, nucleic acids, and proteins (*1*). These tiny vesicles also have been implicated in a number of human diseases, including cancer, and are becoming an appreciated fundamental aspect of tumor progression and metastasis (*2*). Recently, Melo *et al.* (*3*) showed that exosomes from breast cancer cells transfer microRNAs (miRNAs) to normal cells and stimulate them to become cancerous. This potentially expands the mechanisms by which cancer spreads and may provide opportunities to develop exosome-based diagnostics and therapies.



MiRNA biogenesis. MiRNAs combine with AGO2 and other proteins in an RNA-induced silencing complex (RISC) to repress the translation of target mRNAs.



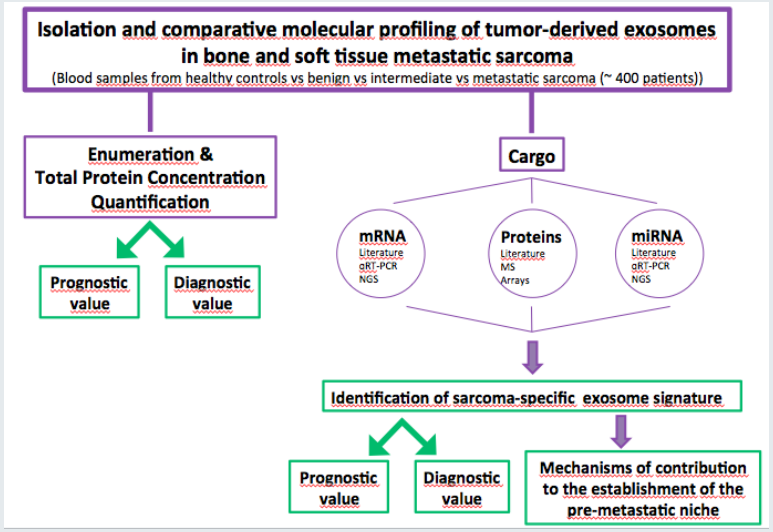
Targeting cancer exosomes. Three possible therapeutic scenarios are shown for targeting tumor-derived exosomes within a cancer cell (**A**), in a normal recipient cell (**B**), or in the circulation (**C**).

→ **Functional Relevance and Potential Use as Diagnostic and Prognostic Factors in Metastatic Sarcoma**

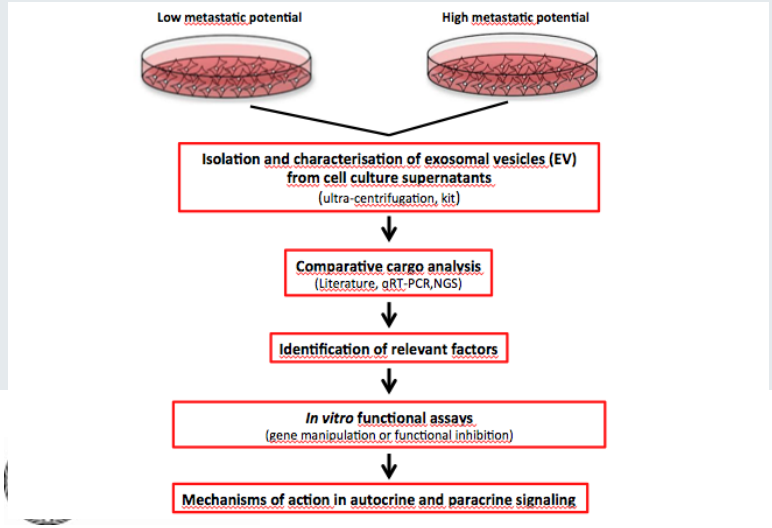
PERSONALIZED MEDICINE

5. Exosomes (Tumor Derived Microvesicles)

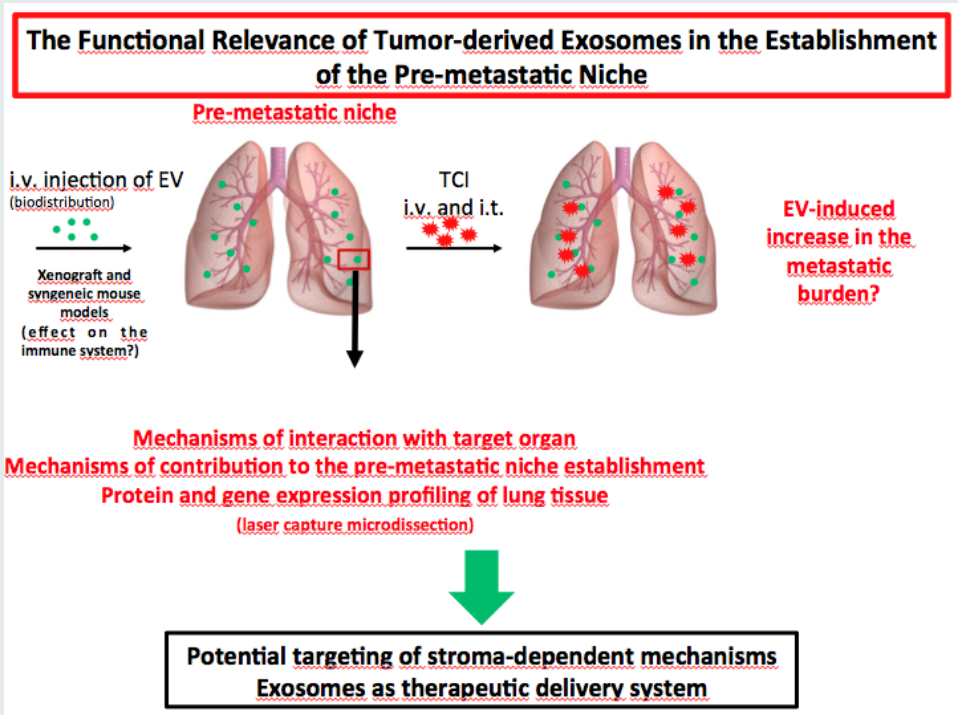
Patients‘ tumor material



In vitro modelling



In vivo modelling



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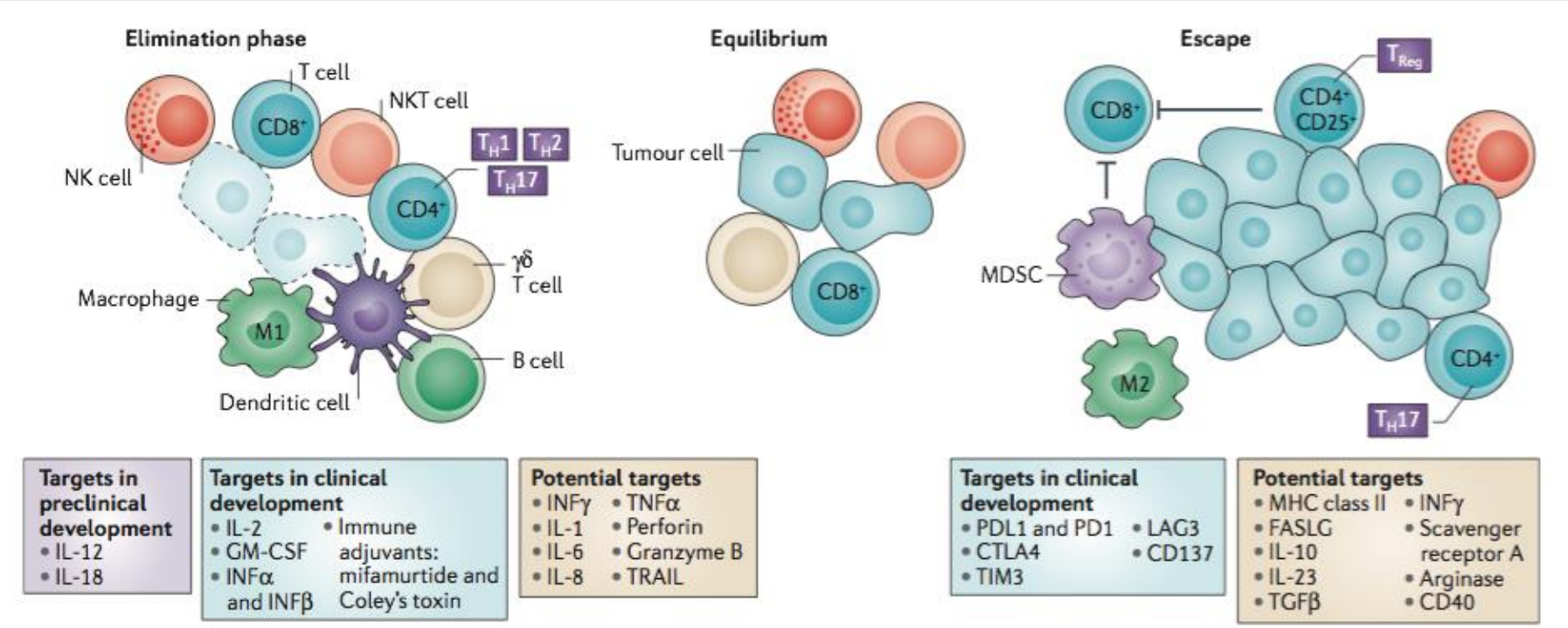
PERSONALIZED MEDICINE
6. Targeting Imunomodulators



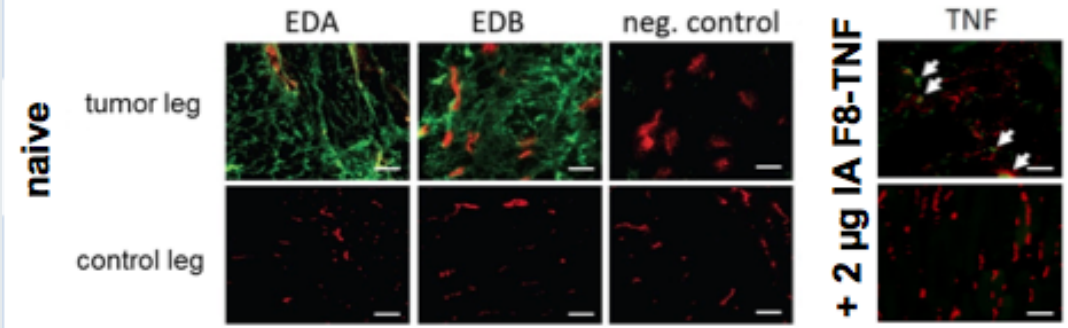
→ Immune checkpoint inhibitors may improve survival

Kansara M et al Nature Rev Cancer 14:722-35, 2014

PERSONALIZED MEDICINE

6. Targeting Imunomodulators

Targets: EDA, EDB



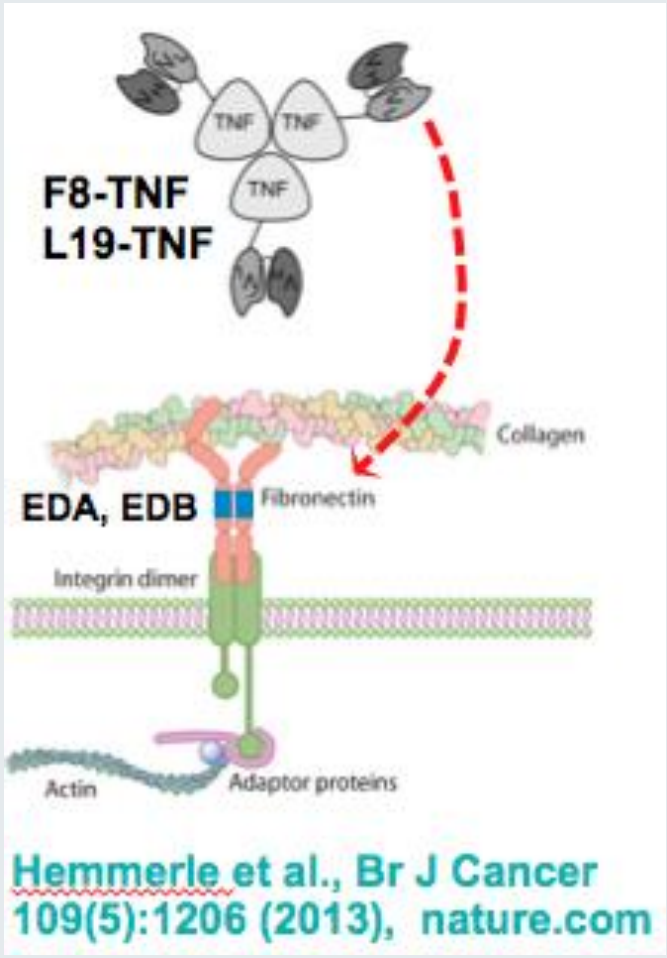
"Immunocytokines"

Pasche & Neri, Drug Discov Today 17(11-12):583 (2012)

- F8-TNF, L19-TNF
- Selective accumulation in sarcoma tumors
- Immunological response
- Combination with doxorubicin: cure

Preclinical testing in OS mouse model

- Single treatment, combination with cisplatin
- IL-2/CTLA4, IL-4, IL-6, IL-13, IL-10, IL-12



Hemmerle et al., Br J Cancer 109(5):1206 (2013), nature.com

AB – mediated Drug Delivery

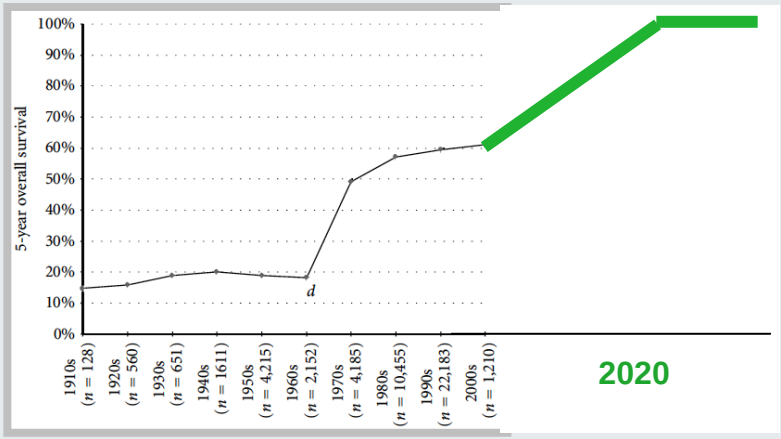
WHERE DO WE GO ?

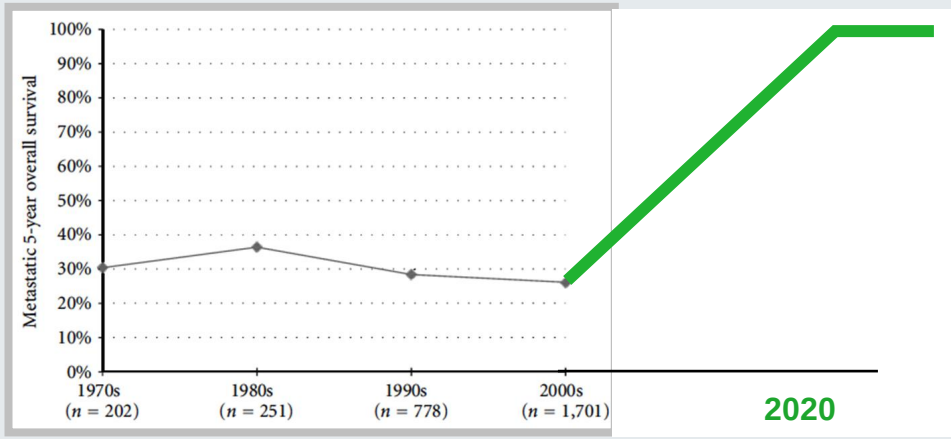
CANCER
IT'S PERSONAL
THE RIGHT PATIENT. THE RIGHT TREATMENT.

→ strong clinical data set (SwissSARCOS)

→ good sarcoma tissue bank (SwissSARCTissues)

→ strong Translational Sarcoma Research Platform





→ *real basis to ↑ survival in the future !*

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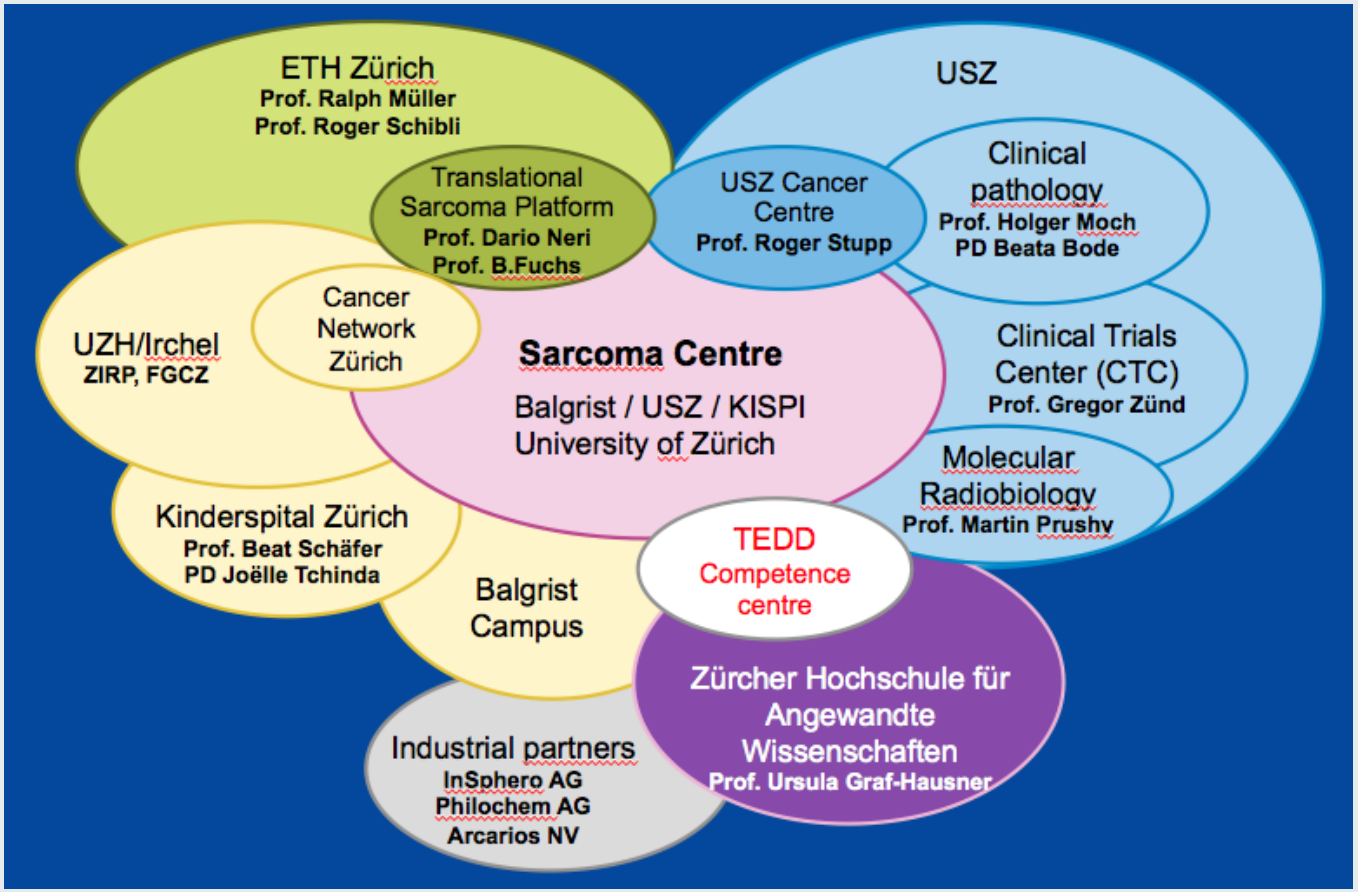
Sarkomzentrum ZÜRICH

THE TRANSLATIONAL SARCOMA RESEARCH PLATFORM



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