Osteosarcoma: Current Perspectives and Future Developments

Bruno Fuchs, MD PhD
OSTEOSARCOMA

Definition:
- malignant spindle cells
- osteoid production

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

M 58%
F 42%
n=1821

Sarkomzentrum Zürich
OSTEOSARCOMA
Backbone of Therapy

- Surgery
- Chemotherapy
- Radiation 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, an international randomised study for osteosarcoma: results from pre-randomisation treatment


Table 2. Pathology at diagnostic biopsy and surgery

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Total: 2209 | 100 | 2012 | 100
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


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.

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 aged ≤ 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 of 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). Baseline characteristics were 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.
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
WHAT IS BEING TARGETED?

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
plateau, unchanged for years!

Metastasis

TARGETED THERAPY - BIOMARKER

WHY DID IT FAIL?
different subtypes of OS!

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

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.

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

REASONS: MICROENVIRONMENT

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

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 Muff1, Prisni Rath2, Ram Mohan Ram Kumar1, Knut Husmann1, Walter Born1, Michael Baudis2, Bruno Fuchs1*

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.

→ cell lines are unstable!

Muff R et al. PlosOne DOI:10.1371; 2015
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!

Uluckan Ö et al BoneKEy Reports 4, 670, 2015
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, Matthias J.E. Artl, Bernhard Robl, Aleksandar Kuzmanov, Walter Born, Bruno Fuchs

*Laboratory for Orthopaedic Research, Department of Orthopaedics, Balgrist University Hospital, University of Zurich, Zurich, Switzerland

Mohan R et al. Am J Cancer Res in publication
WHAT IS BEING TARGETED?

Translational biology of osteosarcoma


→ personalized medicine!
LATEST DEVELOPMENTS

The evolution of cancer

Metastases are seeded by subclones:

a.) 1° Tumor
b.) metastatic sites
c.) polyclonal seeding (same sets of subclones seed multiple sites)

focus on CTC & dormancy ???
LATEST DEVELOPMENTS
CTC & Dormancy

Nature Reviews Cancer

PERSPECTIVES

Metastasis prevention by targeting
the dormant niche

Cyrus M. Chajär

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?

outcome & survival

+ molecular research
  current targeted therapy
+ multidisciplinarity
+ one discipline
wait & see

→ how do we invest?
QUEST FOR PERSONALIZED MEDICINE

diagnostic markers
improved imaging
individualized therapy
→ prognosis ↑
QUESTS FOR PERSONALIZED MEDICINE

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

3 Major Areas

Cooperation
Clinical Data
- Local, national and international level

Patient material based research
- Patient tissue xenograft (PDX) models
- Drug testing

therapy/diagnostics Imaging/monitoring
- Quantitative treatment monitoring (experimental, clinical)
A. Definition of Advisory Board / Sarcoma Boards / Sarcoma Centers

B. Guidelines

C. cohort study

D. research – tissue collection

www.sarcoma.ch
Swiss National Sarcoma Advisory Board

Sarcoma Center

(accorded according to international guidelines)

- Sarcoma-Board
  - Supraregional Tertiary Referral Center (Cyberfish)
- Sarcoma Research
  - Translational Sarcoma Platform
- Sarcoma Cohort
  - SwissSarcos
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

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

> 200 patients from one single center
COHORT STUDY
SWISS SARCOS

Data Management

Sarkomzentrum A

data system of local institution zB Kisim

Sarkomzentrum B

Sarkomzentrum C

Sarkomzentrum D

Sarkomzentrum F

Sarkomzentrum E

ethic approval obtained!

www.sarcoma.ch
TISSUE COLLECTION

Knowledge:
drug targets & biomarkers

Functions &
mechanism of action

Analysis

Cancer
genomics

Drug and biomarker
discovery and development
Genomics-informed clinical trials
Regulatory and
commercial challenges

Patient consents
Sample acquisition
Clinical annotation
Study design

Sarkomzentrum Zürich

Universität Zürich
TISSUE COLLECTION
Working Group

What to collect

- Patient material
  - Blood
    - 1A) CTC
    - 1B) Whole blood & WBC
  - Serum
  - Plasma
  - Tissue
    - 4A), D) Metabolites Proteins DNA RNA
    - 4B) Pathol. Protein (IHC)
  - Cell culture
  - Protein (Western) DNA (Sequencing, CGH) RNA (microarray)

Open questions

- Priority for tissue (4A, B, C, D)?
- 1B) not performed yet (to be discussed)
- 4D) not performed yet (to be discussed)
- Decentralized storage, centralized registration in database?
- Centralized database read only?
- Who updates registration?
- Who decides which samples can be used for studies?
- Anonymization (no PID)?

www.sarcoma.ch
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
MOUSE MODELLING OF OS
Orthotopic Sarcoma Mouse Models

MOUSE MODELLING OF OS
LacZ tagging of individual cells

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

MicroSprayer Aerosolizer®

Neklyodova O et al. In preparation
PERSONALIZED MEDICINE
Where do we focus on?

→ several areas of interest!
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. atypical 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

miRNAs as discriminators in liposarcoma
### Personalized Medicine

#### 1. Patient Stratification

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<td>1 24.12.2014</td>
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<td>y Lungs, Spleen, Bone</td>
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36 patients in total; MLS: 13; L: 10; ALT: 7; DDLS: 5; PML: 1

---

**miRNAs as discriminators in liposarcoma**
miRNAs as discriminators in liposarcoma

correlation of miR155 expression in plasma & tumor samples

PERSONALIZED MEDICINE
1. Patient Stratification

Boro et al, in preparation
**PERSONALIZED MEDICINE**

1. **Patient Stratification**

miRNAs as discriminators in liposarcoma

Boro et al, in preparation
An *in vitro* osteosarcoma 3D microtissue model for drug development

Markus Rimann\(^a,1\), Sandra Laternser\(^a,1\), Ana Gvozdenovic\(^b\), Roman Muff\(^c\), Bruno Fuchs\(^d\), Jens M. Kelm\(^e\), Ursula Graf-Hausner\(^h,1\)

\(^a\) Institute of Chemistry and Biological Chemistry (ICBC), Zurich University of Applied Sciences, Winterthur, Switzerland
\(^b\) Laboratory for Orthopaedic Research, Department of Orthopaedics, University of Zurich, Switzerland
\(^c\) inSphero AG, Schlieren, Switzerland

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**PERSONALIZED MEDICINE**

2. *in-vitro* 3D & *in-vivo* modelling

**Standard treatment**

- Biopsy
- Diagnosis
- Chemotherapy
- Resection
- Chemotherapy

**Future Improved treatment**

- 3D microtissues from biopsy

---

**Reliable 3D cell culture platform**

- 2D and *in vivo* data
- Gene expression data

---

**Paving the way to personalized medicine**

- Biobank
- Clinical patient information
- Tumor tissues
- Xenograft models
- Tissue analysis
- Genome and Proteome profiling
- *In vitro* 3D models

---

**Personalized patient treatment**

- Clinical OS research
- *In vitro* and *in vivo* OS models
- Biobank

---

**Drug sensitivity**

---

**Patient’s tumor material**
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!
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

Arlt M et al, Sarcoma 2013 ID 404962
3. Dormancy

Lenti Vector for MSH043018-1-LVRH1MP

Bhlhb9 overexpression & knockdown

\[2^{-\Delta\Delta C_t}\]

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<th>LM8-LZ</th>
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</table>

mCherry Co-expression

\[\text{in vivo experiments underway!}\]
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!
● 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!
**Malicious exosomes**

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

By Elena Anastasiadou and Frank J. Slack

Exosomes are membrane components and cytoplasmic contents of these cells that play an important role in intercellular communication often inducing physiological changes in target cells by transferring biomolecules such as lipids, nucleic acids, and proteins. These tiny vesicles also have been implicated in a number of human diseases, including cancer, and are becoming an appreciated tool in the understanding of tumor progression and metastasis. Recently, Li et al. 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.

**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

- **Isolation and comparative molecular profiling of tumor-derived exosomes in bone and soft tissue metastatic sarcoma**
  - Blood samples from healthy controls vs benign vs intermediate vs metastatic sarcoma (~400 patients)

- **Enumeration & Total Protein Concentration Quantification**
- **mRNA**
  - Literature
  - qPCR
  - NGS
- **Proteins**
  - Literature
  - SDS-PAGE
- **mRNA**
  - Literature
  - qPCR
  - NGS

- **Identification of sarcoma-specific exosome signature**
- **Prognostic value**
- **Diagnostic value**

### In vivo Modelling

- **The Functional Relevance of Tumor-derived Exosomes in the Establishment of the Pre-metastatic Niche**
- **Pre-metastatic niche**
  - i.v. injection of EV (distribution)
  - Xenograft and syngeneic mouse model
  - Effect on the immune system?

- **Mechanisms of interaction with target organ**
- **Mechanisms of contribution to the pre-metastatic niche establishment**
- **Protein and gene expression profiling of lung tissue**
  - Laser capture microdissection

- **Potential targeting of stroma-dependent mechanisms**
- **Exosomes as therapeutic delivery system**

### In vitro Modelling

- **Low metastatic potential**
- **High metastatic potential**

- **Isolation and characterisation of exosomal vesicles (EV) from cell culture supernatants**
  - Ultra-centrifugation, is }

- **Comparative cargo analysis**
  - (Literature, qPCR, NGS)

- **Identification of relevant factors**

- **In vitro functional assays**
  - (Gene manipulation or functional inhibition)

- **Mechanisms of action in autocrine and paracrine signalling**

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Sarkomzentrum Zürich
PERSONALIZED MEDICINE

6. Targeting Immunomodulators

→ Immune checkpoint inhibitors may improve survival

PERSONALIZED MEDICINE

6. Targeting Immunomodulators

Targets: EDA, EDB

“Immunocytokines“
- 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

AB – mediated Drug Delivery
WHERE DO WE GO?

→ strong clinical data set (SwissSARCOS)

→ good sarcoma tissue bank (SwissSARCTissues)

→ strong Translational Sarcoma Research Platform

→ real basis to ↑ survival in the future!
THE TRANSLATIONAL SARCOMA RESEARCH PLATFORM

Sarcoma Centre
Balgrist / USZ / KISPI
University of Zürich

ETH Zürich
Prof. Ralph Müller
Prof. Roger Schibli

USZ
USZ Cancer Centre
Prof. Roger Stupp

Clinical pathology
Prof. Holger Moch
PD Beata Bode

Clinical Trials Center (CTC)
Prof. Gregor Zünd

Molecular Radiobiology
Prof. Martin Prushy

UZH/Irchel ZIRP, FGCZ
Cancer Network Zürich

Kinderspital Zürich
Prof. Beat Schäfer
PD Joëlle Tchinda

Balgrist Campus

TEDD Competence centre

Zürcher Hochschule für Angewandte Wissenschaften
Prof. Ursula Graf-Hausner

Industrial partners
InSphero AG
Philochem AG
Arcarios NV
Acknowledgements

Financial support:

Der Balgrist

Fonds National Suisse
Schweizerischer Nationalfonds
Fondo Nazionale Svizzero
Swiss National Science Foundation

Universität Zürich

HSM MSKO Program
Zürich

Walter L. & Johanna Wolf Foundation
THANK YOU!

bruno.fuchs@balgrist.ch

www.sarkomzentrum.ch