

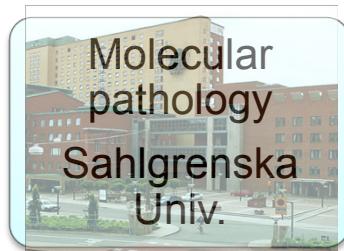
Techniques for sarcoma diagnostics

Molecular Pathology of Solid Tumors
Uppsala 120924

Klinisk Molekylär Patologi

Klinisk Patologi
och
Cytologi





Analyses

Gene Tumor

PDGFRA

GIST

KRAS

Colon

Lung

BRAF

Papilliar Thyreoidea

Malign Melanoma

EGFR

Lung

RET

MEM2

TCR

Lymfoma

IgH

Lymfoma

Fusion genes

Sarcoma

Lymfoma

Lung

HER2

Breast

Venticle

Microsatellit repeat

KIT

GIST

Malign Melanoma

Urovysion



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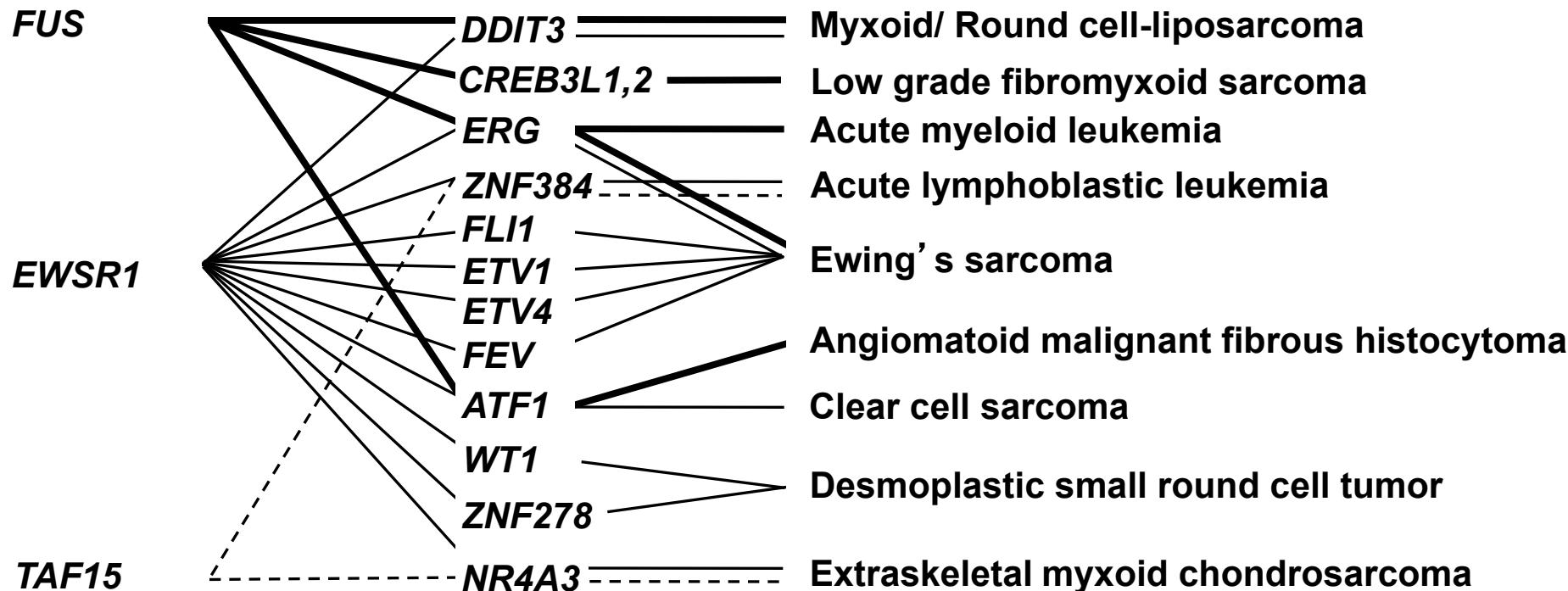
Malign Melanoma

Urovysion

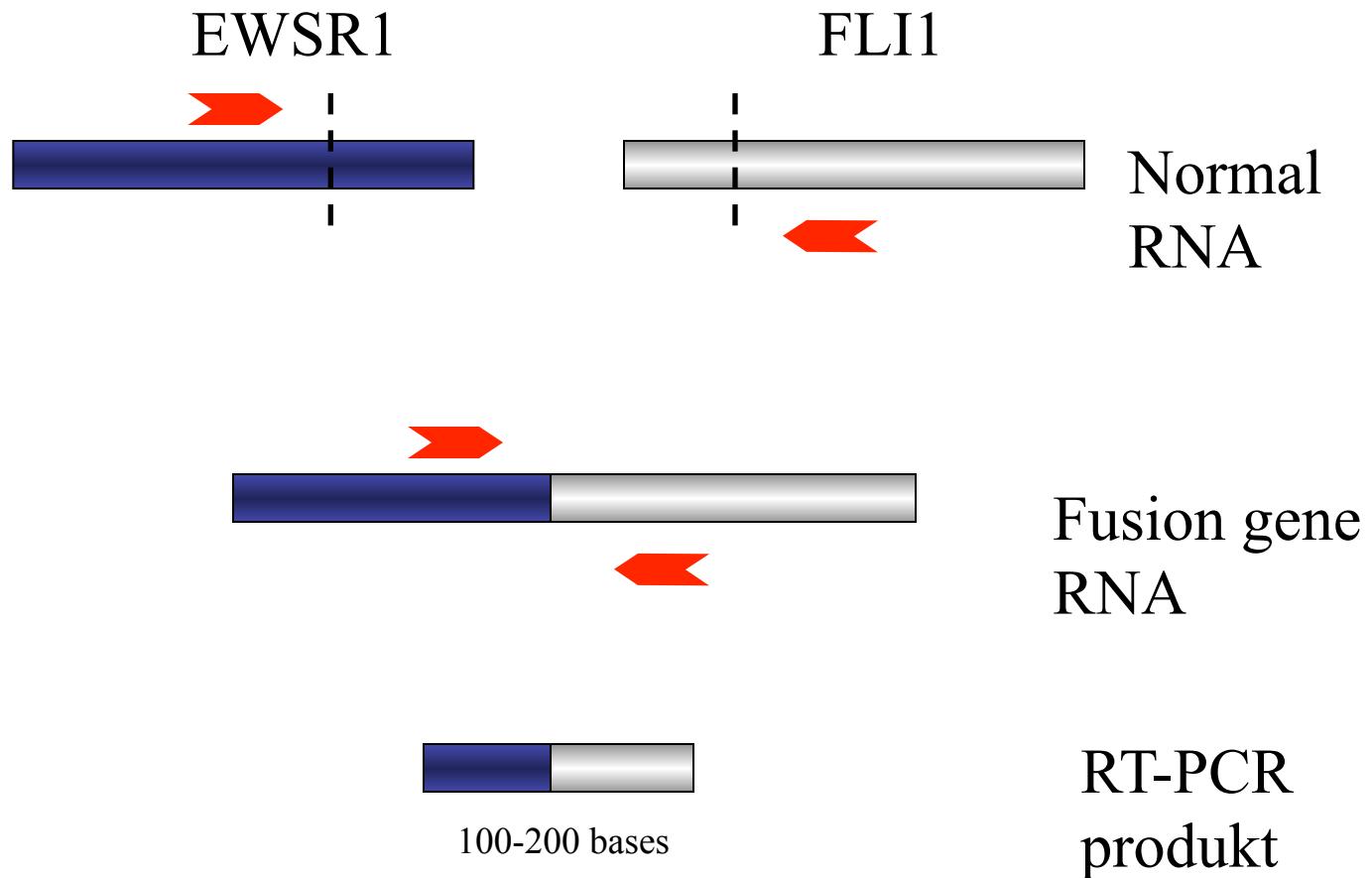
Chromosomal translocations in disease...



Fusion genes are often tumor specific and therefore of diagnostic value

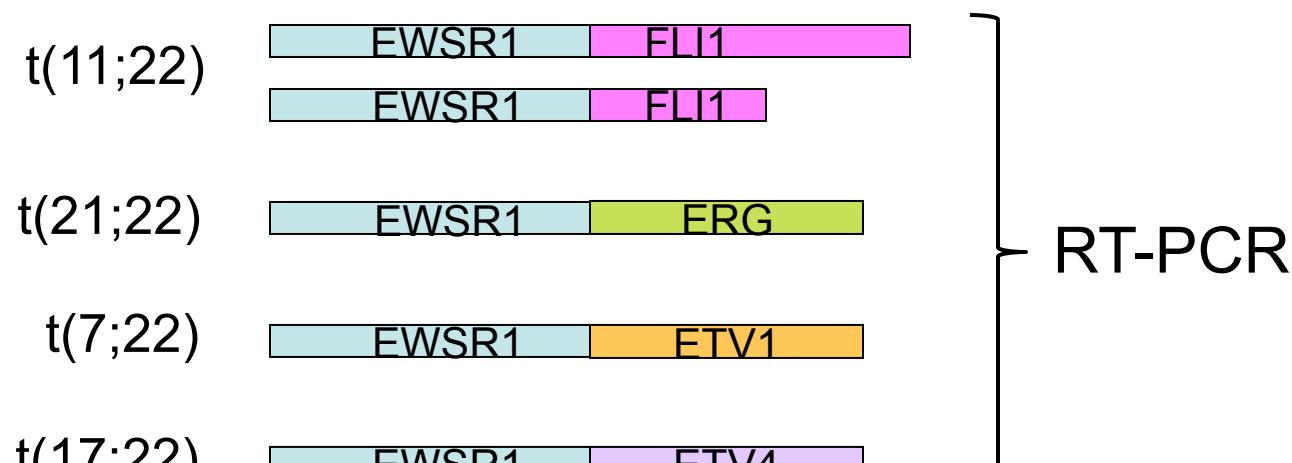


Fusion genes



One sarcoma, many transcripts

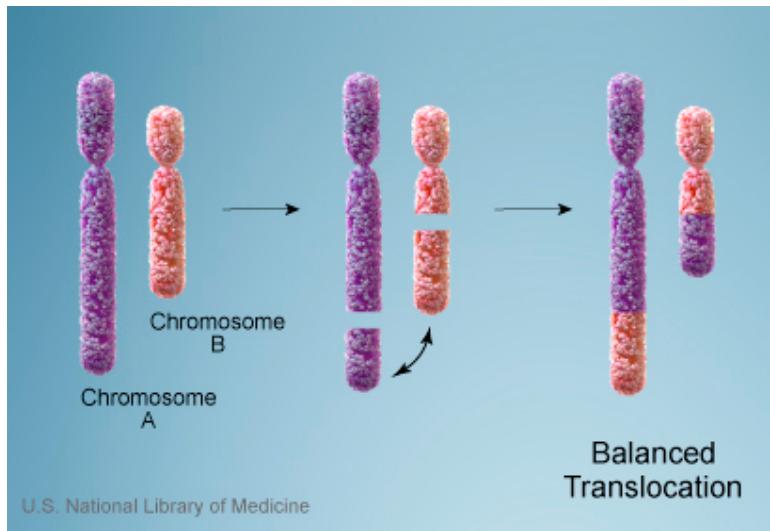
Example: Ewing sarcoma



Kromosom 22  } FISH

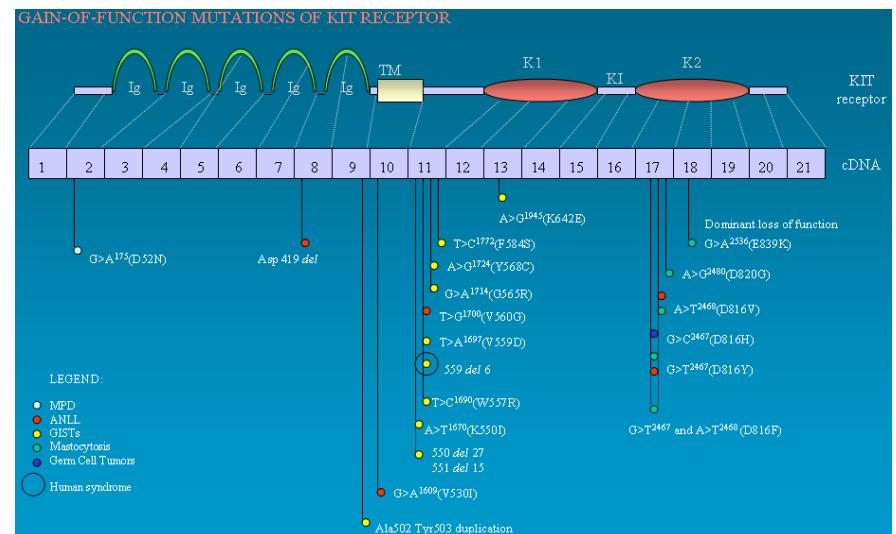
Chromosomal translocation vs mutation detection

Sarcoma



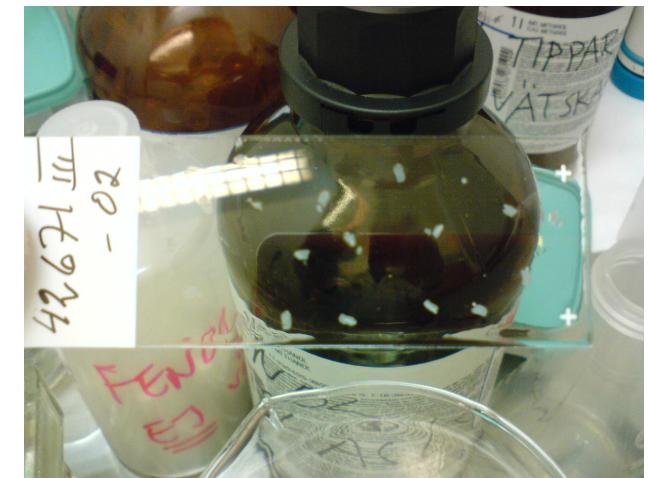
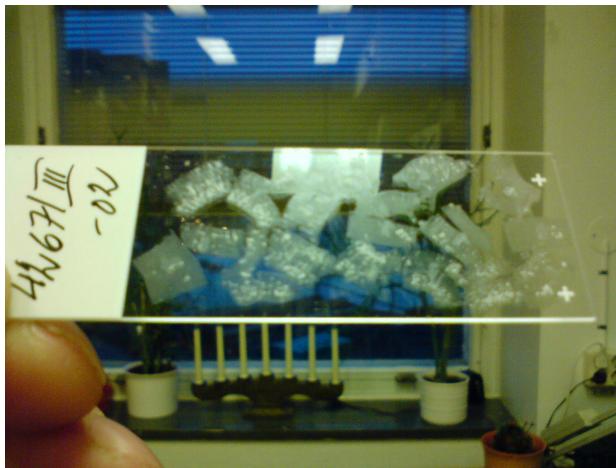
FISH RT-PCR (RNA)
 "tumor specific"

GIST (Kit ex 9,11,13)



DNA
 In all cells (heterogeneity)

Laboratory practice...



Middle needle biopsies

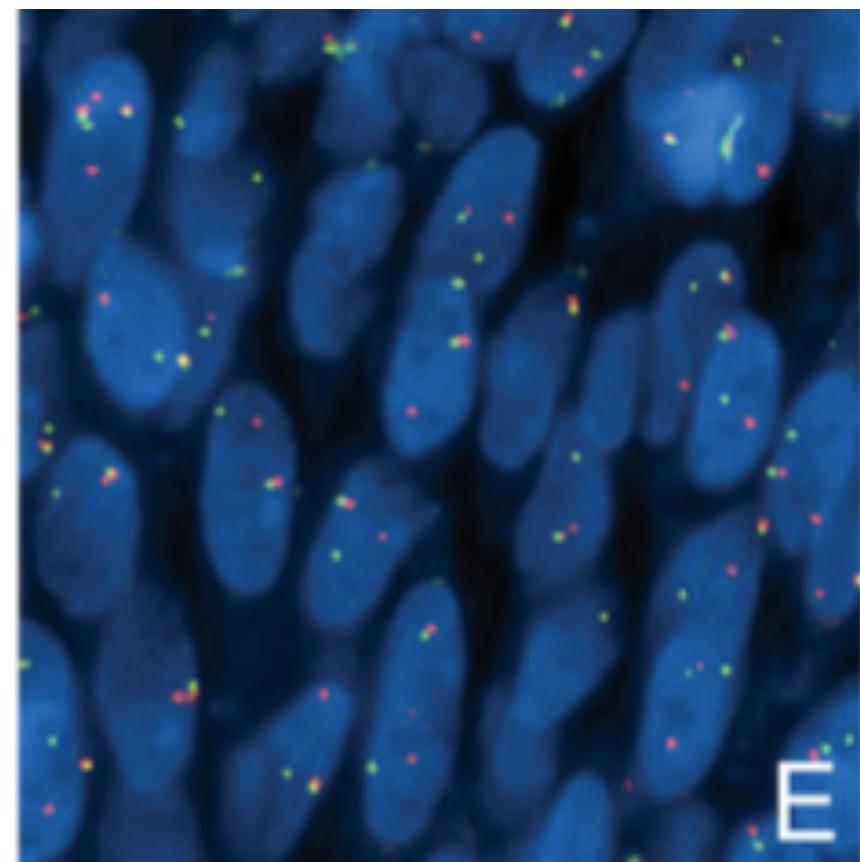
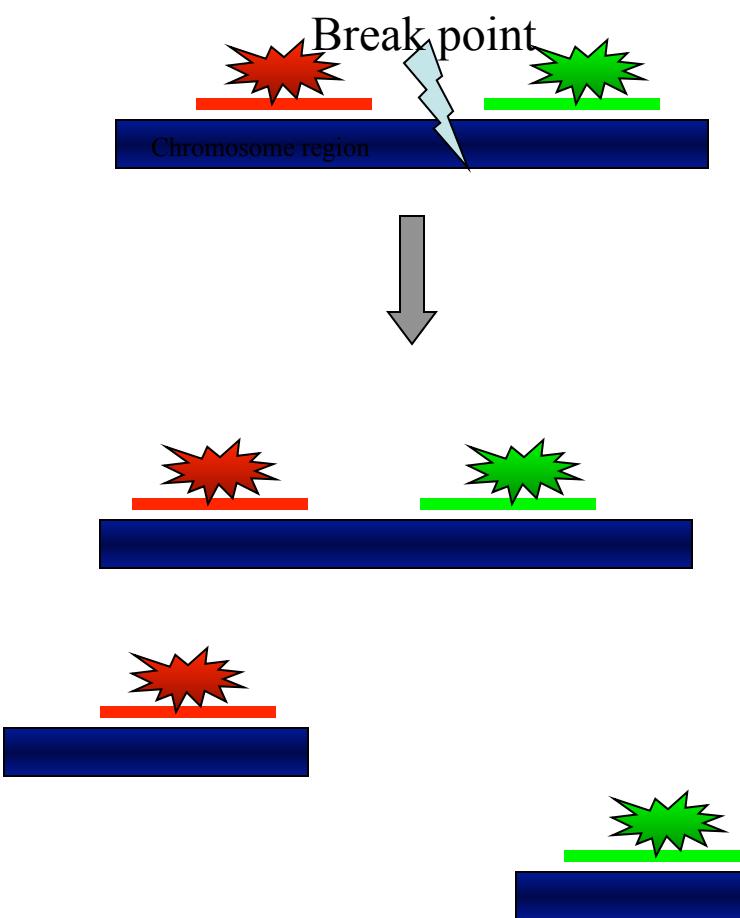


Initial FISH
and then
RT PCR (confirming)



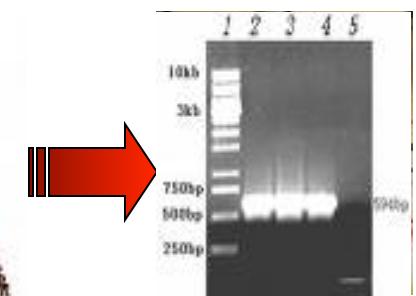
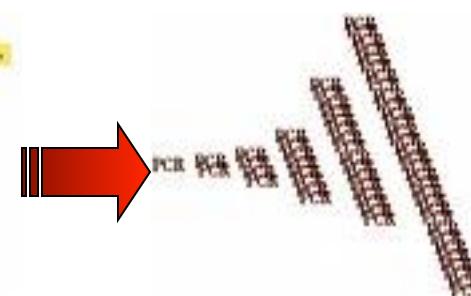
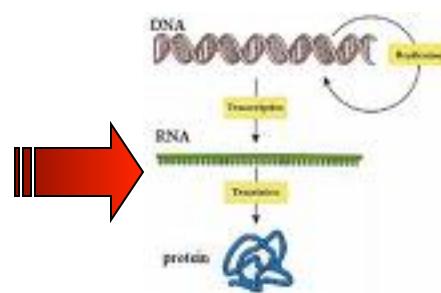
FISH-technique

“split signal”/ “break-a-part”



2x100 cells counted

RT-PCR technique



Paraffin material

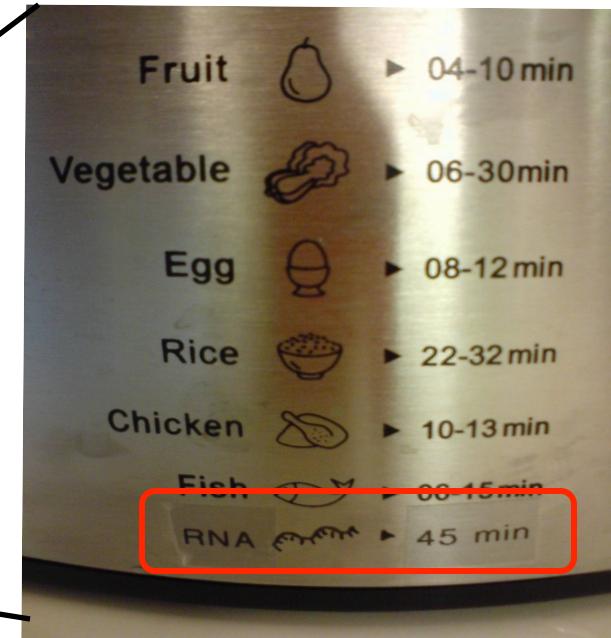
RNA → cDNA

PCR

Gel
separation

RNA-extraction

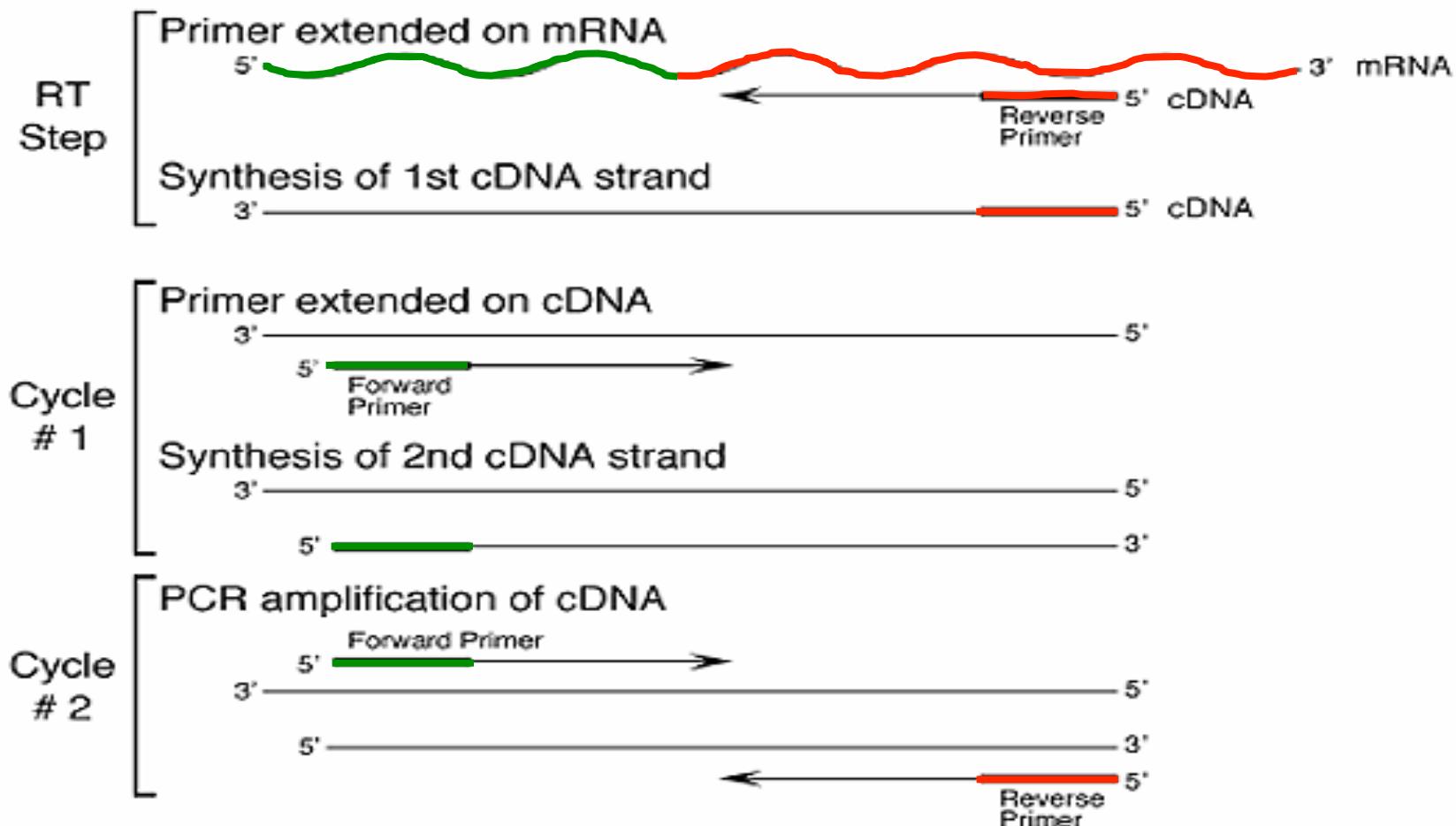
The old way... “RNA steamer”



Today, RNeasy FFPE-kit
(Qiagen)

Targeted OneStep RT-PCR

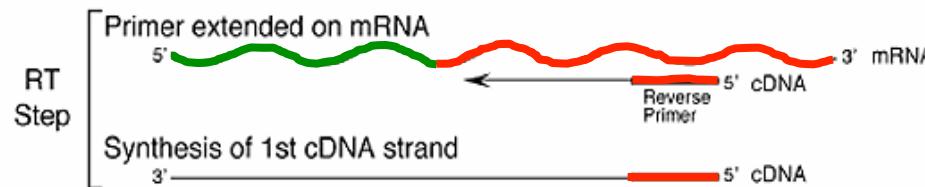
FLI1-primer Reverse



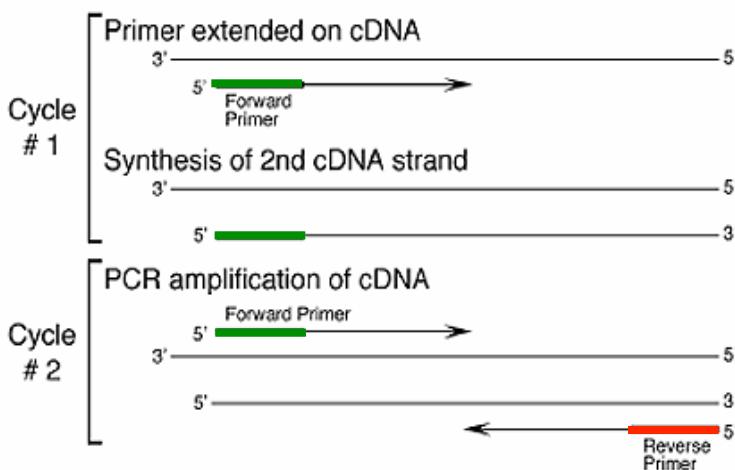
EWS-primer Forward

Targeted OneStep RT-PCR

FLI1-primer Reverse



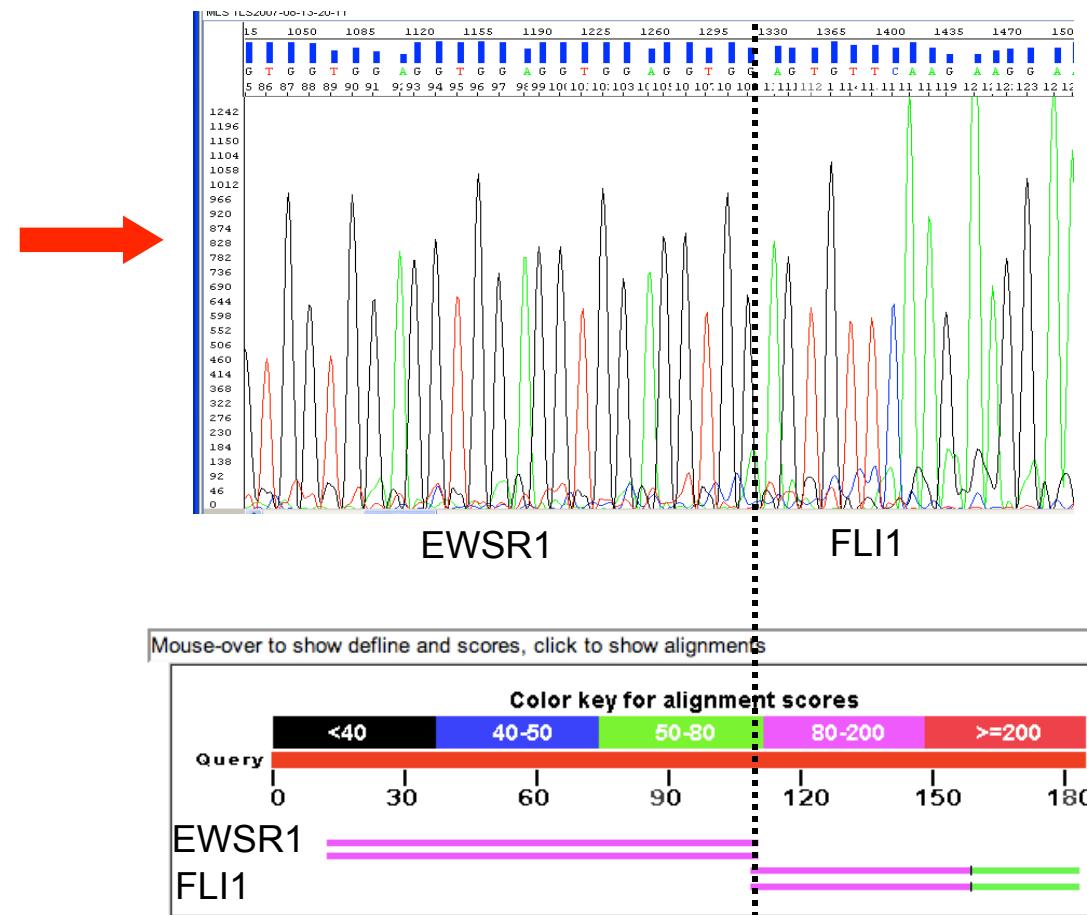
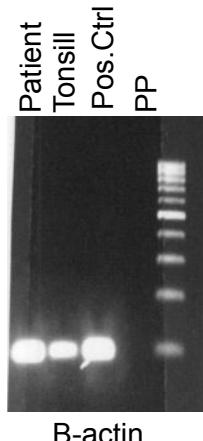
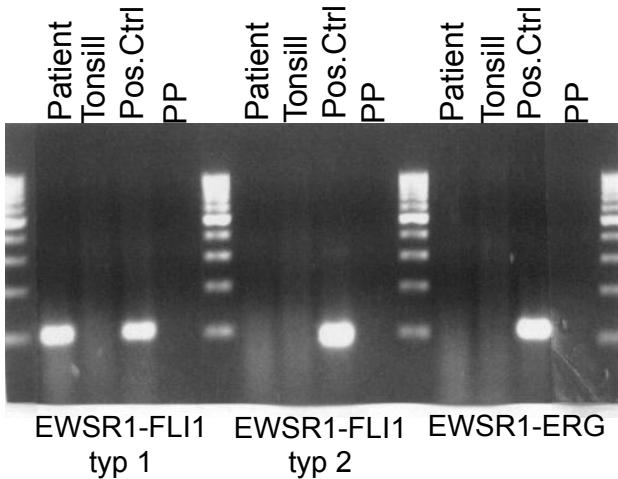
- Gene specific
 - Higher sensitivity
 - Increased amplification



EWS-primer Forward

One Onestep per transcript variant

Result

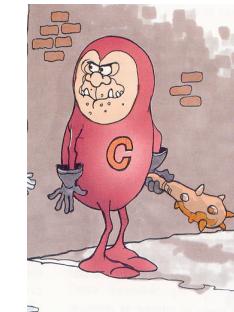


Fusion genes in diagnostics at KMP, Sahlgrenska University hospital

- **EWSR1-FLI1 typ 1/2** Ewingsarkom
- **EWSR1-ERG** Ewingsarkom
- **SYT-SSX typ 1/2** Synovialsarkom
- **EWSR1-ATF1** Clearcellssarkom
- **EWSR1-CHN/TEC** Extraskeletal Myxoidchondrosarkom
- TAF2N-CHN Extraskeletal Myxoidchondrosarkom
- **FUS-DDIT3 typ 1/2** Myxoidliposarkom/Rundcellsliposarkom
- **EWSR1-DDIT3** Myxoidliposarkom/Rundcellsliposarkom
- **EWSR1-WT1** Desmoplastisk small round cell sarkom
- **PAX3-FOXO1A (FKHR)** Alveolär Rhabdomyosarkom
- **PAX7-FOXO1A (FKHR)** Alveolär Rhabdomyosarkom
- **ETV6-NTRK3** Congenital fibrosarkom
- **COL1A1-PDGFB** Dermatofibrosarkom protuberans (DFSP)

(FISH-probes)

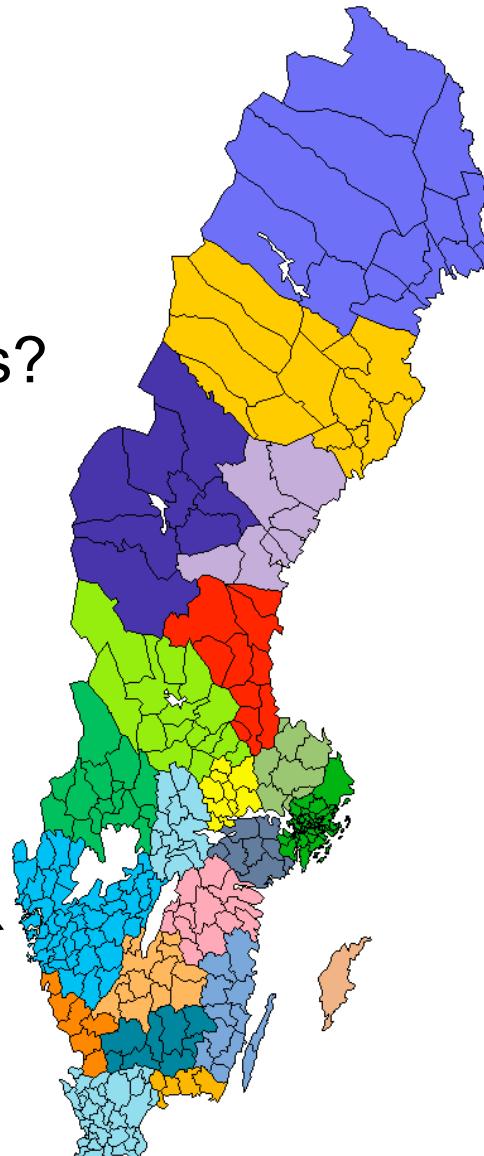
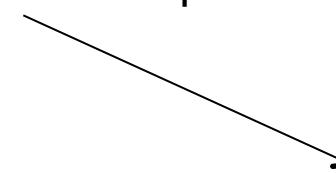
2012-10-16



Fredrik.Enlund@gu.se

Centralisation for Ewing sarcoma diagnostics?

Sahlgrenska Univ. Hosp.
Göteborg

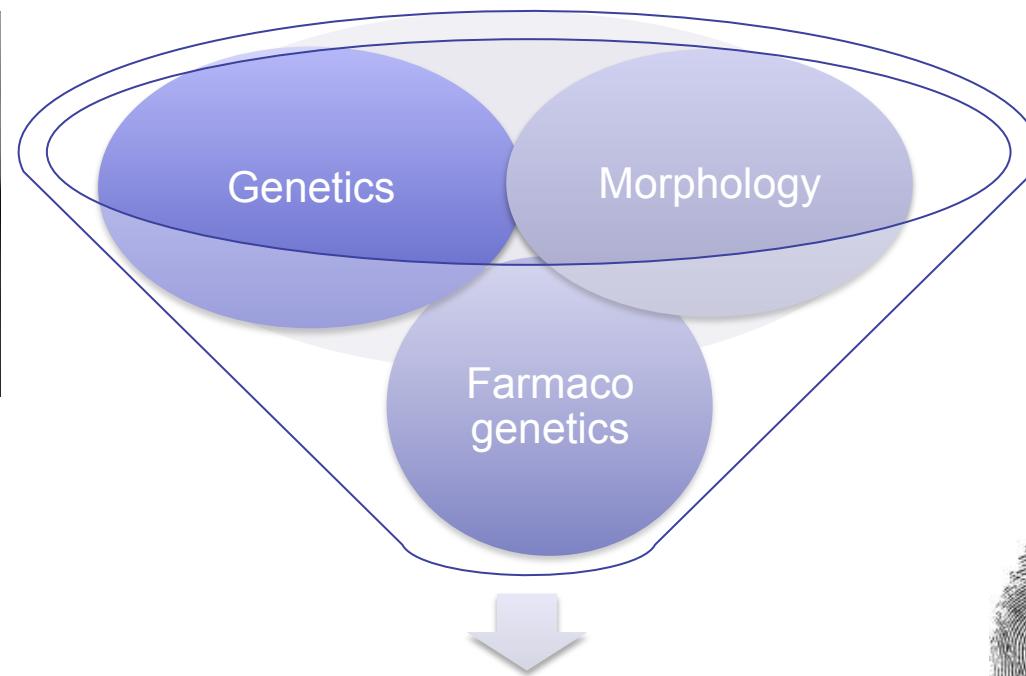


R&D project in Sarcoma

first and second approach

Our future challenge

The **right drug** for the **right patient** at the **right dose**

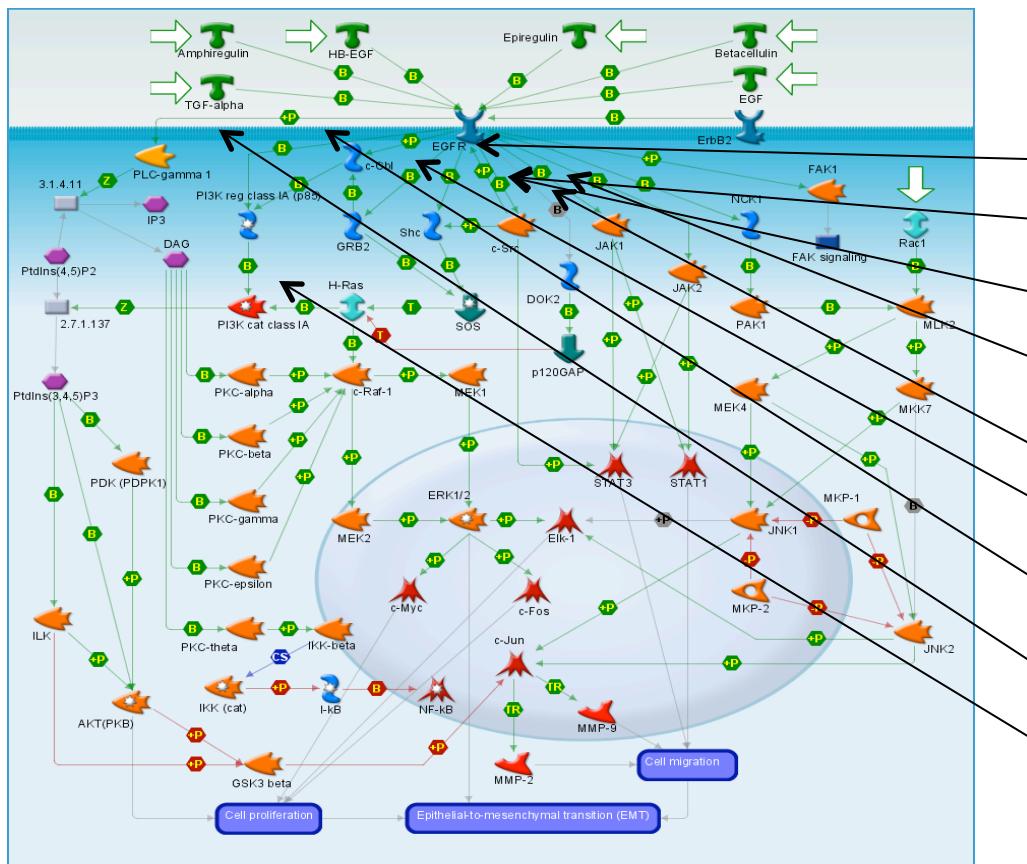


Individually tailored medication
BIOMARKER(S)



It's in our genes...

Aim: To look for well known biomarkers for targeted treatment in sarcoma



Target genes

EGFR1
BRAF
NRAS
KRAS
cKIT
PDGFRA
JAK2
HER2
PIK3

Enhanced mutation detection

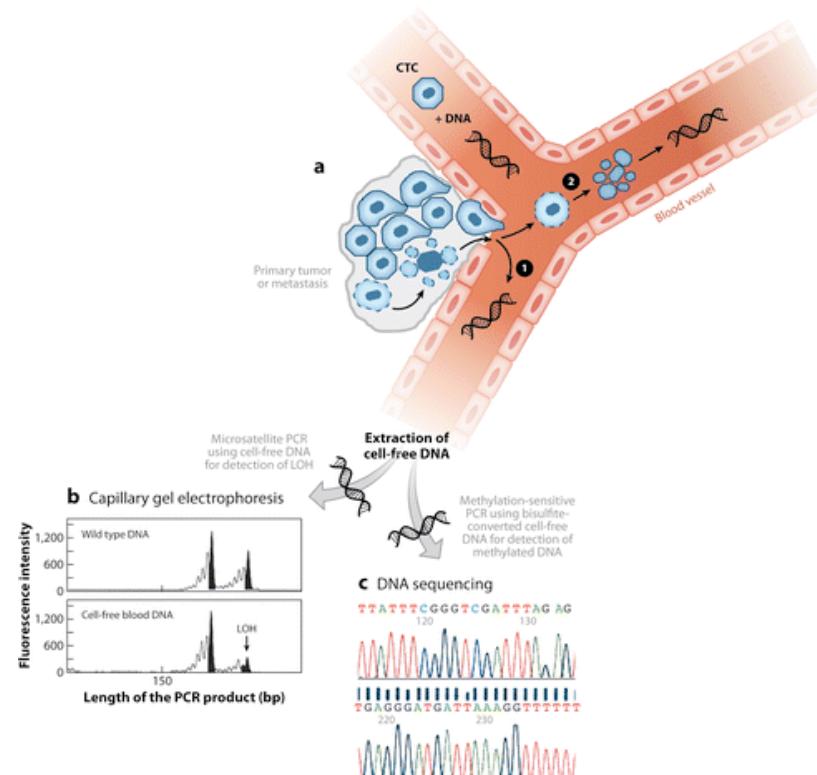
1st approach

- When available – ARMS or sensible techniques
- Macro dissection of tumor material
- Material from # 39 FFPE Ewing Sarcomas

2nd approach...

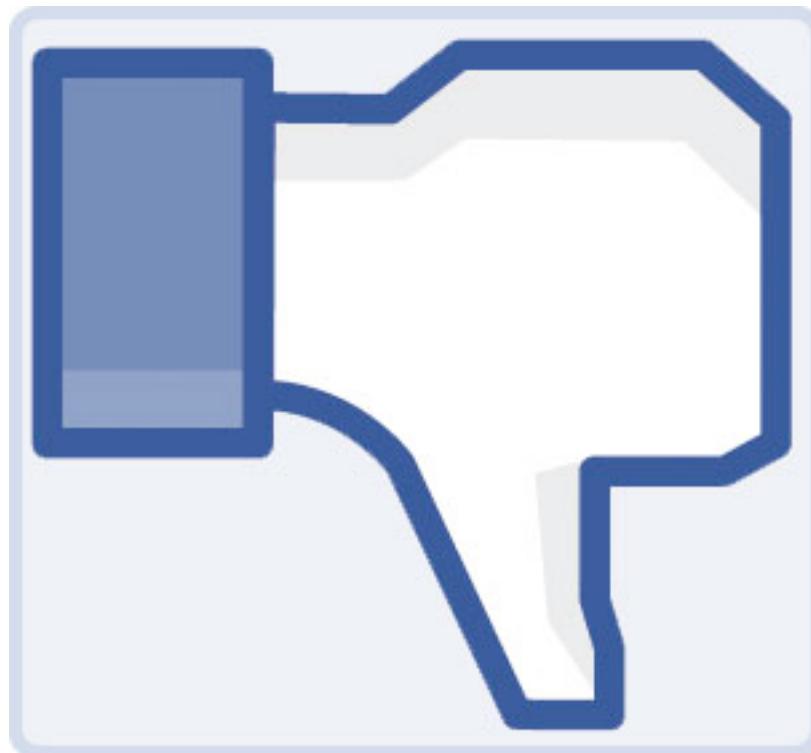
Aim: To Study Circulation Tumor Cells (CTC) in Ewing sarcoma patients

2nd approach



 Alix-Panabières C, et al. 2012.
Annu. Rev. Med. 63:199–215

Disappointing...



1st approach

No significant mutations
detected in none of the
analysed tumors...

(maybe not surprising – fusion genes)

2nd approach

Not completed

Lack of other "knorrs" at the end



Co-workers at KMP,Sahlgrenska?

- 
- Carola Andersson
 - Josefina Andersson
 - Anders Edsjö
 - Tina Ekviden
 - Carina Karlsson
 - Helene Sjögren
 - Anita Viskari

R&D sponsored by "Johan Janssons forskningsstiftelse för sarkom"

Thanks for your attention!

