Sample collection and processing

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Disclosure slide

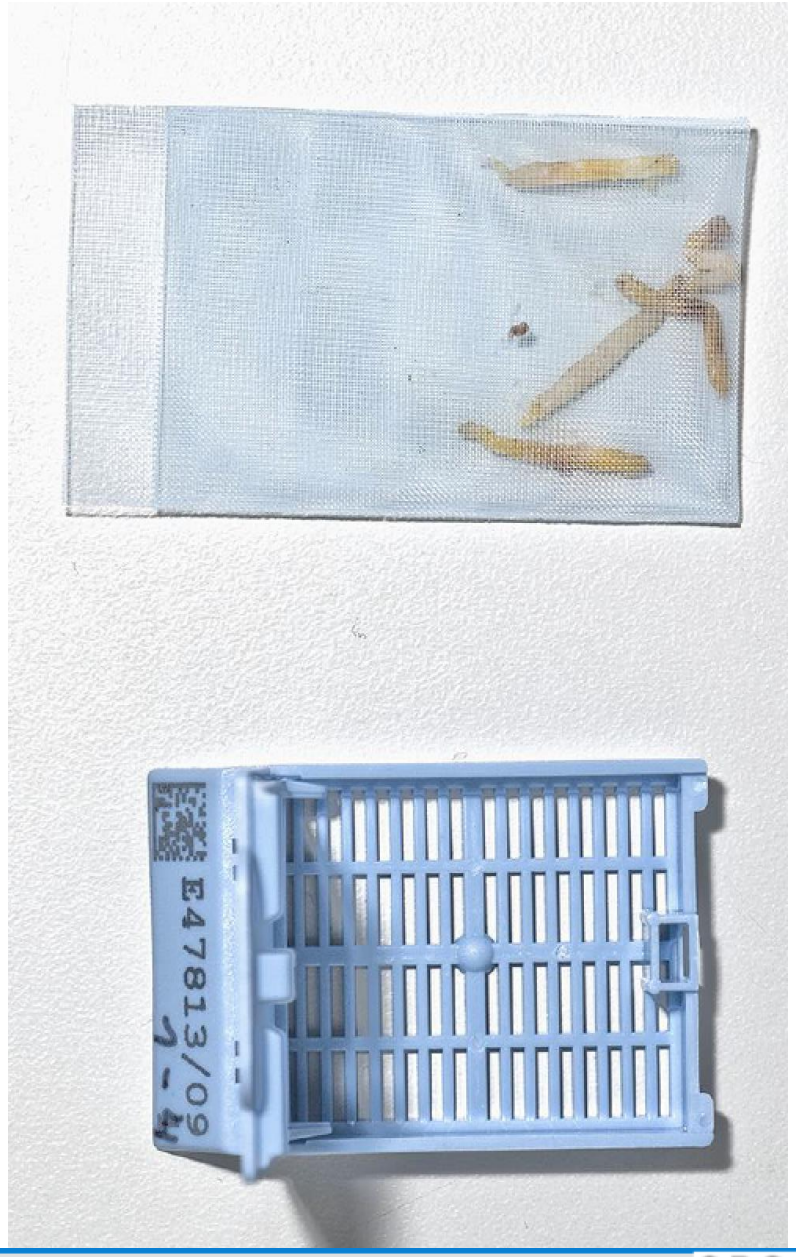
- I am shareholder and cofounder of Sividon Diagnostics.

Outline – sample collection and processing

1. Sample processing for non-pathologists
   - FFPE tissue, H&E, immunohistochemistry
2. sample collection – how to collect as many samples as possible
   - the GBG experience

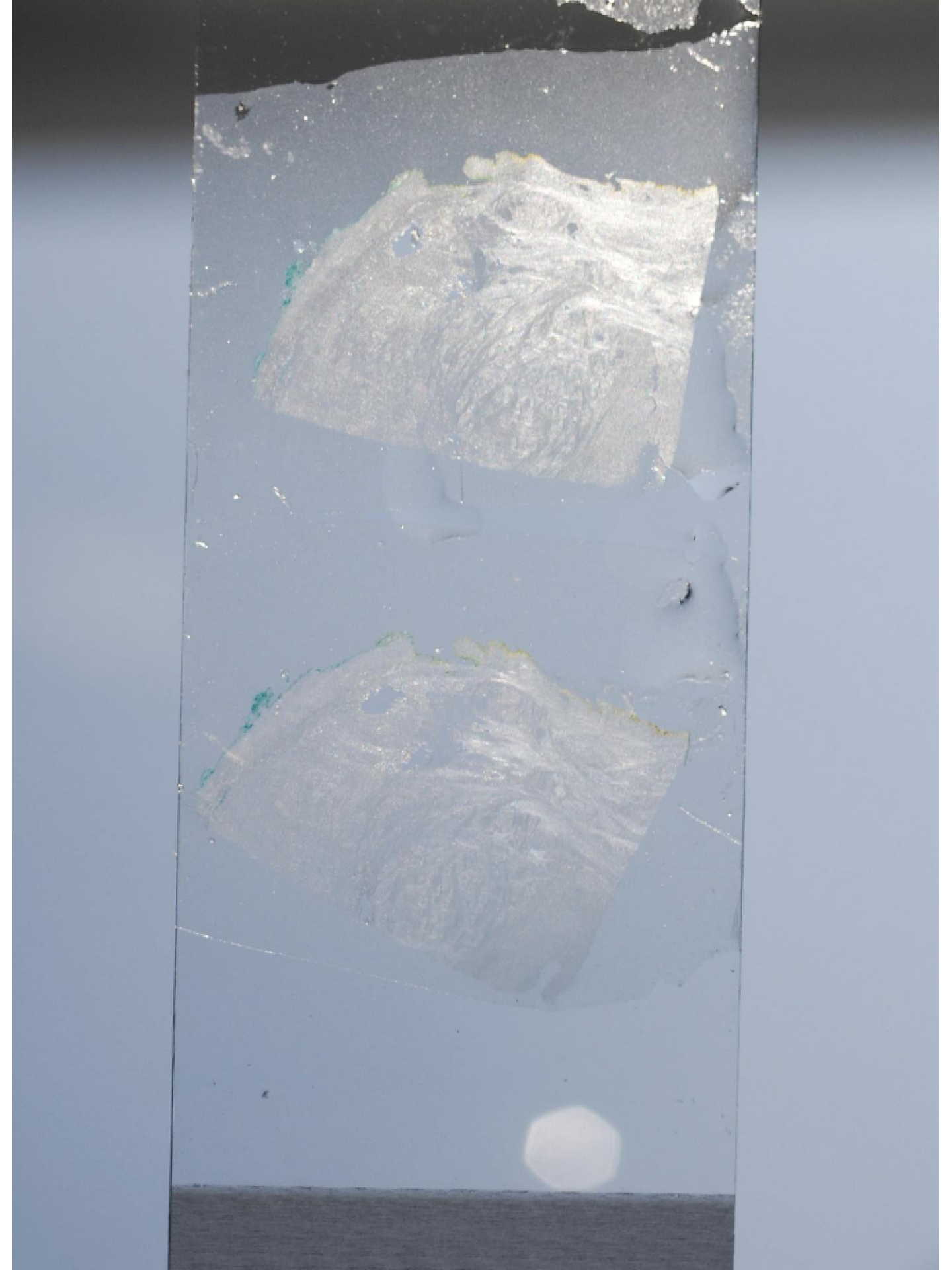
Good communication with pathologists will be central for success of translational research in clinical trials!

Breast cancer core biopsy

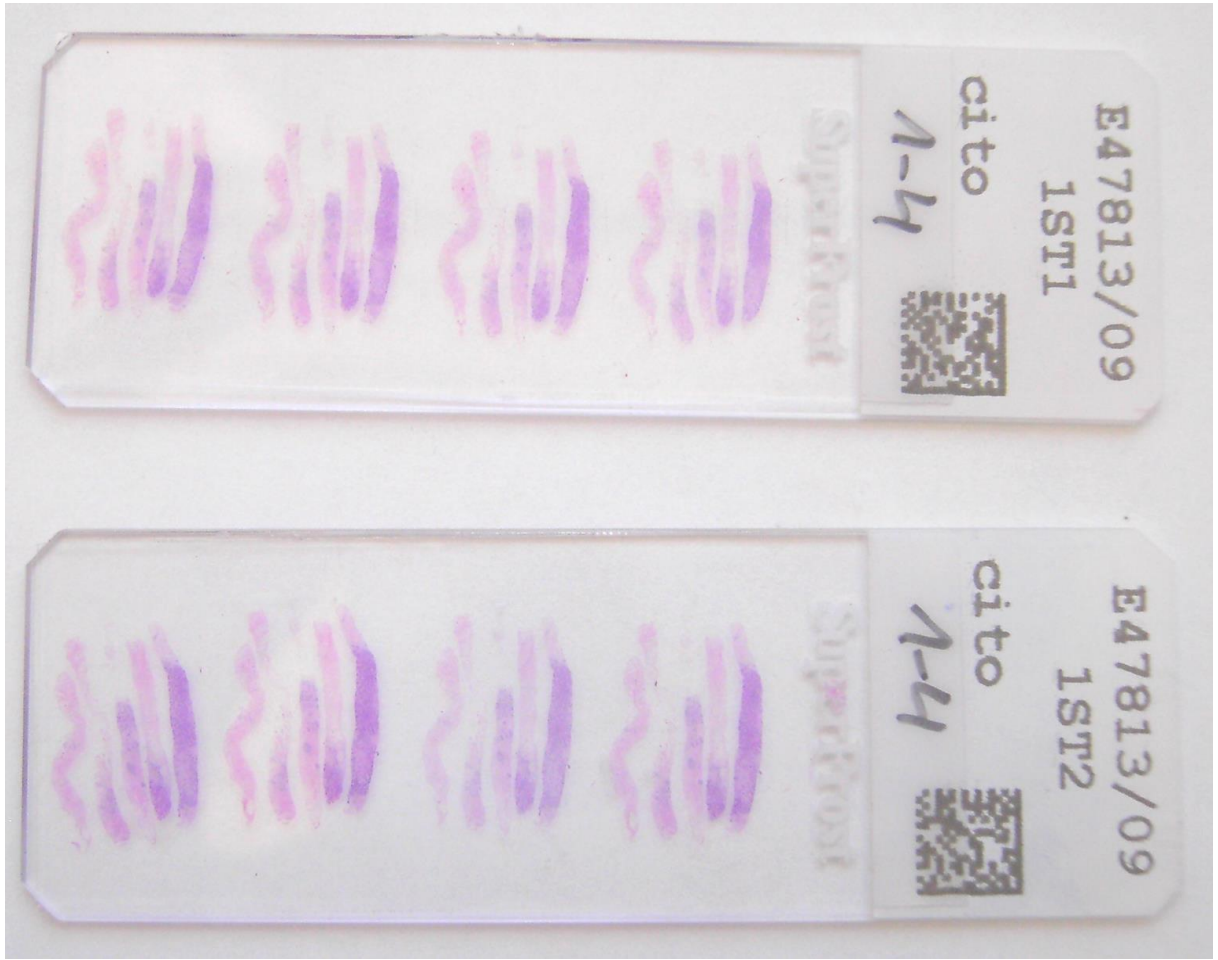


Samples should not be stored in liquid formalin for more than 72h.
Volume of formalin should be at least 10x of the tissue volume.
Never use NaCl.

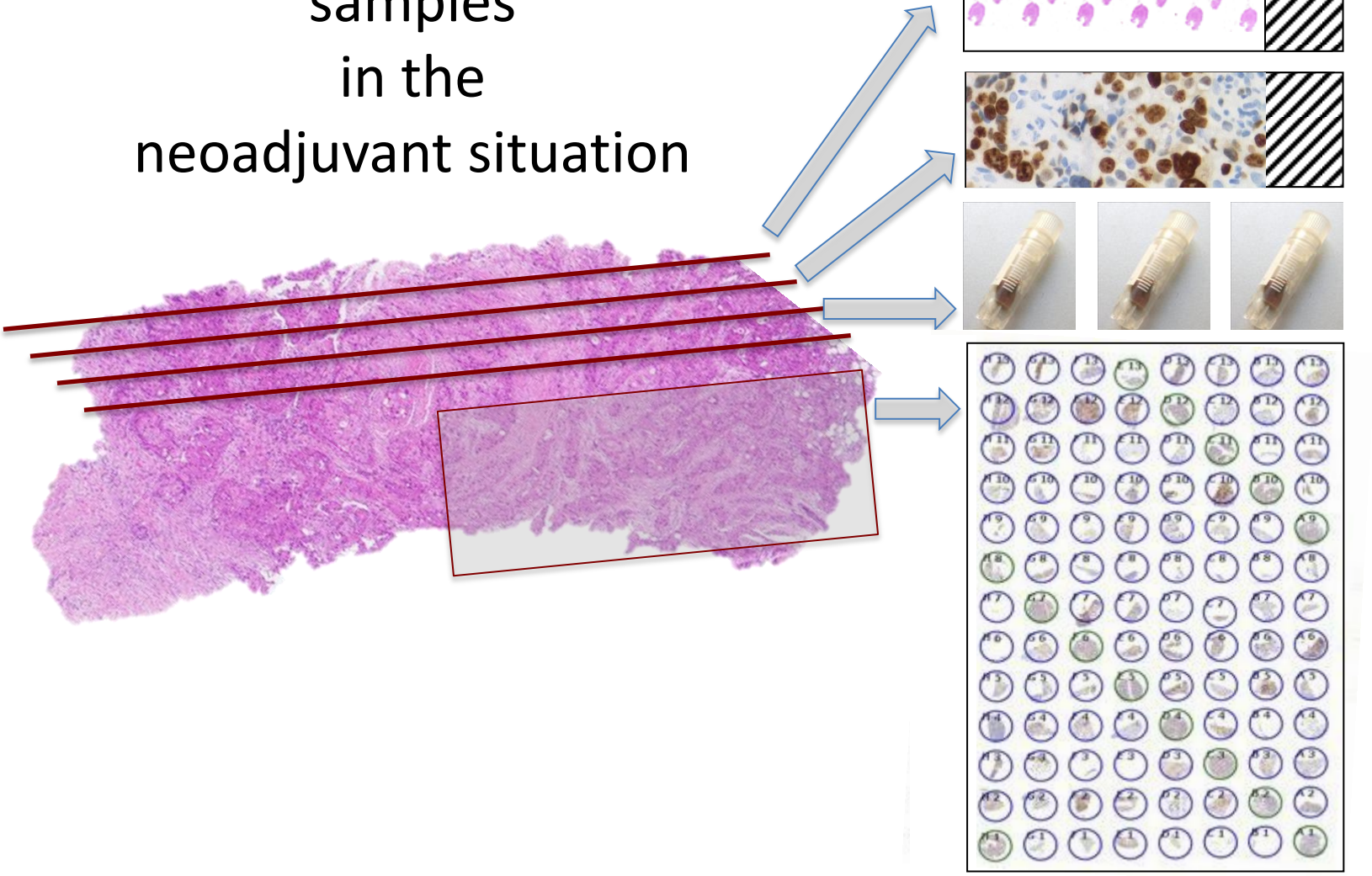

section
thickness 1-
4um

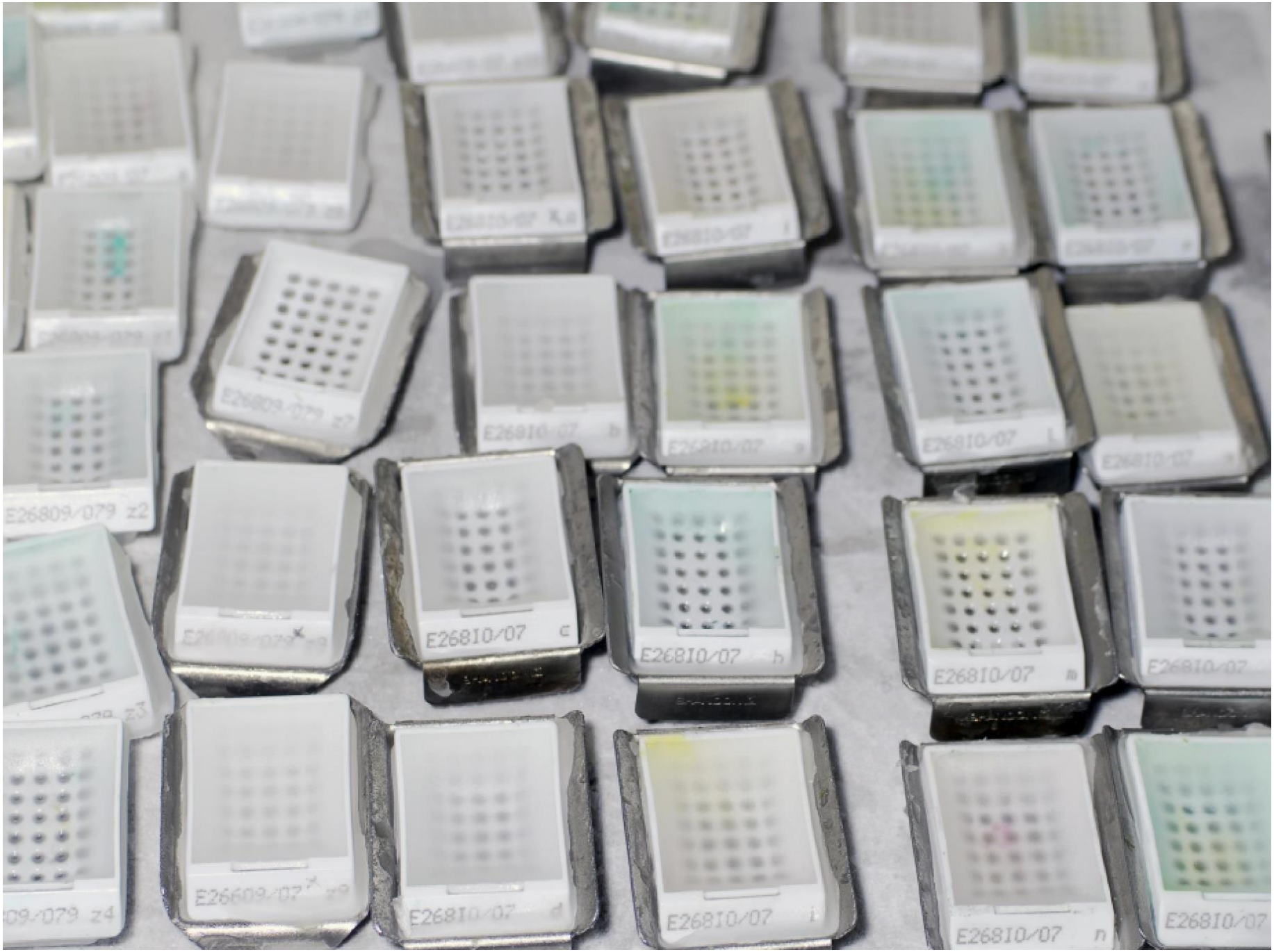


Core biopsy: 1mm (1000um)
H&E (8 sections): 16um
IHC (4 sections): 8um
total: 24um
left for research: 976um



Efficient workflow: Tumor samples in the neoadjuvant situation




E26809/079 z2
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E26810/07 d
E26810/07 i
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Zutreffendes Ankreuzen

E47812-09

Fadenmarkiertes Mammaamputat ○ *rechts*/ ⊗ *links*

Größe: 125 X 130 X 35 mm, **Gewicht** 155 g
Hautspindel: 70 X 30 X ..... mm /subkutane ME ○
○ Op-Wunde/ ○ Narbe ..... mm im ○ oberen ○ unteren/ ○ äußeren ○ inneren Q.
Mamille: 20 mm i.D.
Areola 20 mm i.D.

post inf. Faszie

Schnittfläche:	**1. Befund**	**2. Befund**
Art	① 1-3 + Stanzk.	4-9
Quadrant	lnnen/Unterh.	zentral → Einreichung d. Faszie
Größe		
Ant-Post	6	18 mm
Sup-Inf	6	30 mm
Med-Lat	6	33 mm

Abstand von 1 zu 2 5 mm

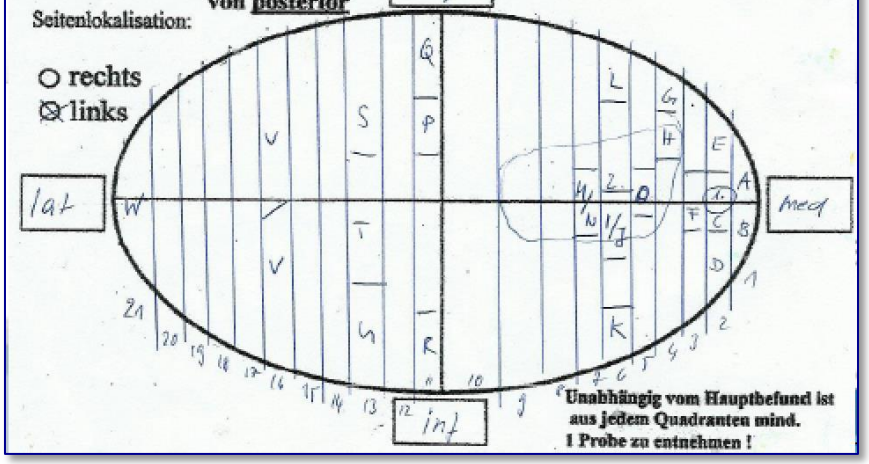
RR	Abstand zum RR in mm		Farbe des RR
Ant	3	0	blau
Post	1	0	grün
Sup	35	25	schwarz
Inf	8	40	gelb
Med	< 5	> 20	
Lat	> 100	> 70	

Kappen auf thi. RR

Blockverteilung: Ansicht von posterior

Seitenlokalisation:

○ rechts
⊗ links



Unabhängig vom Hauptbefund ist aus jedem Quadranten mind. 1 Probe zu entnehmen !

Important issues for different types of research samples

core biopsy

- small sample
- standardized processing = high quality
- only one block = easy to identify
- material for many biomarker assays
- handling of samples is critical
- research biopsies could be collected – careful control is neccessary, logistics my be challenging

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surgical sample

- more material
- fixation and processing less standardized and difficult to control
- many blocks for one specimen: block identification might be challenging
- separate research blocks are feasible

Collection of samples for research -

How can we use tissue samples from histopathology for research projects?

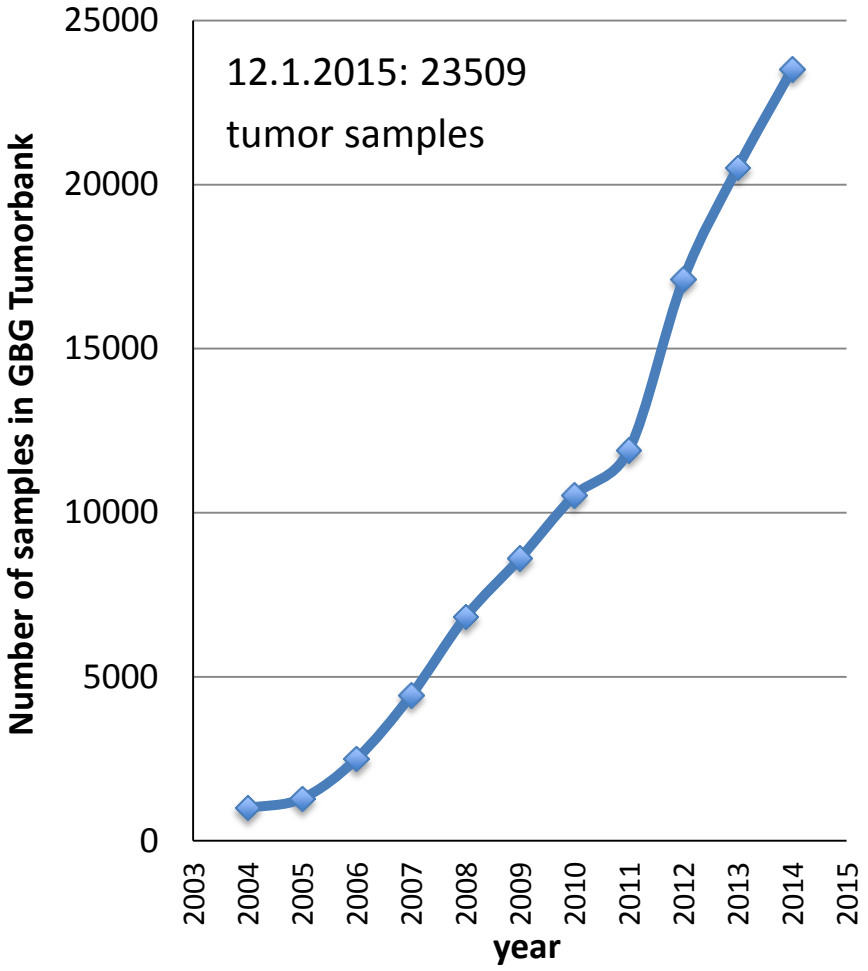
Advantages

- tissue has already been collected
- no additional logistics
- FFPE tissue is stable and suitable for many analyses
- large sample collections are possible

Disadvantages

- tissue might be completely used for research
- pathologist are sometimes reluctant to provide the tissue
- some analysis are not possible with FFPE tissue

GBG biobank – samples from >20.000 patients = 2-3% of all breast cancers in Germany



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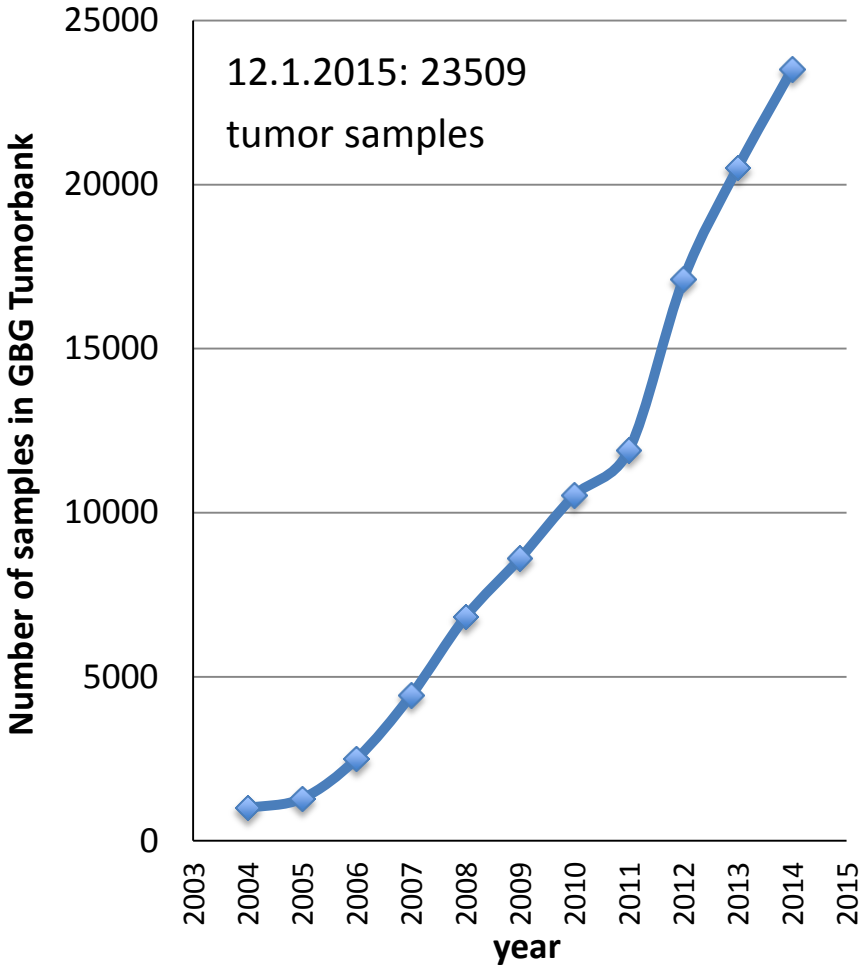
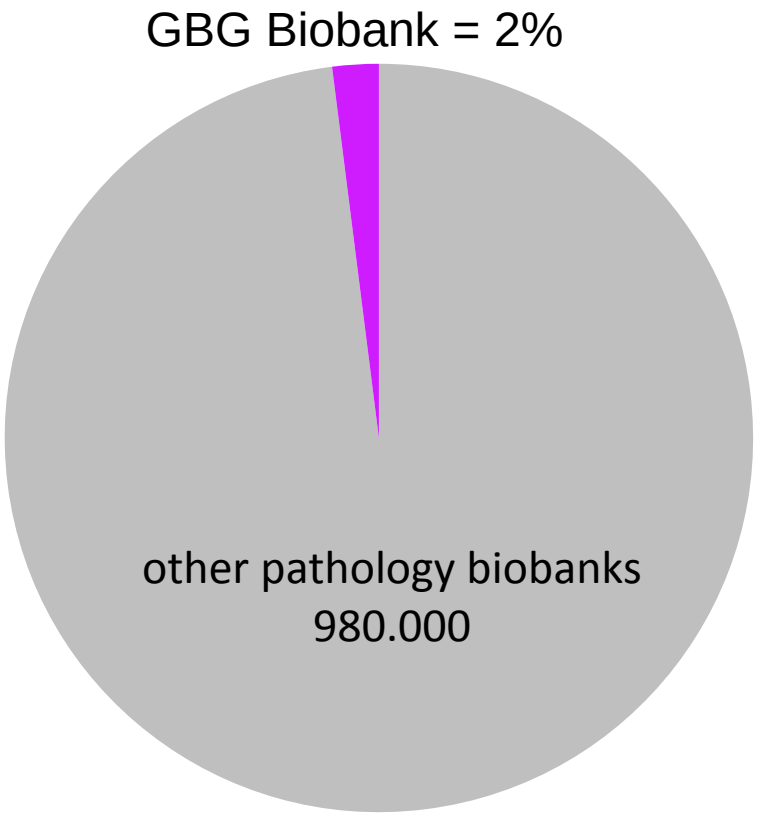


1 Mio. breast cancer diagnoses in Germany 2001-2014



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ASCO SPECIAL ARTICLE

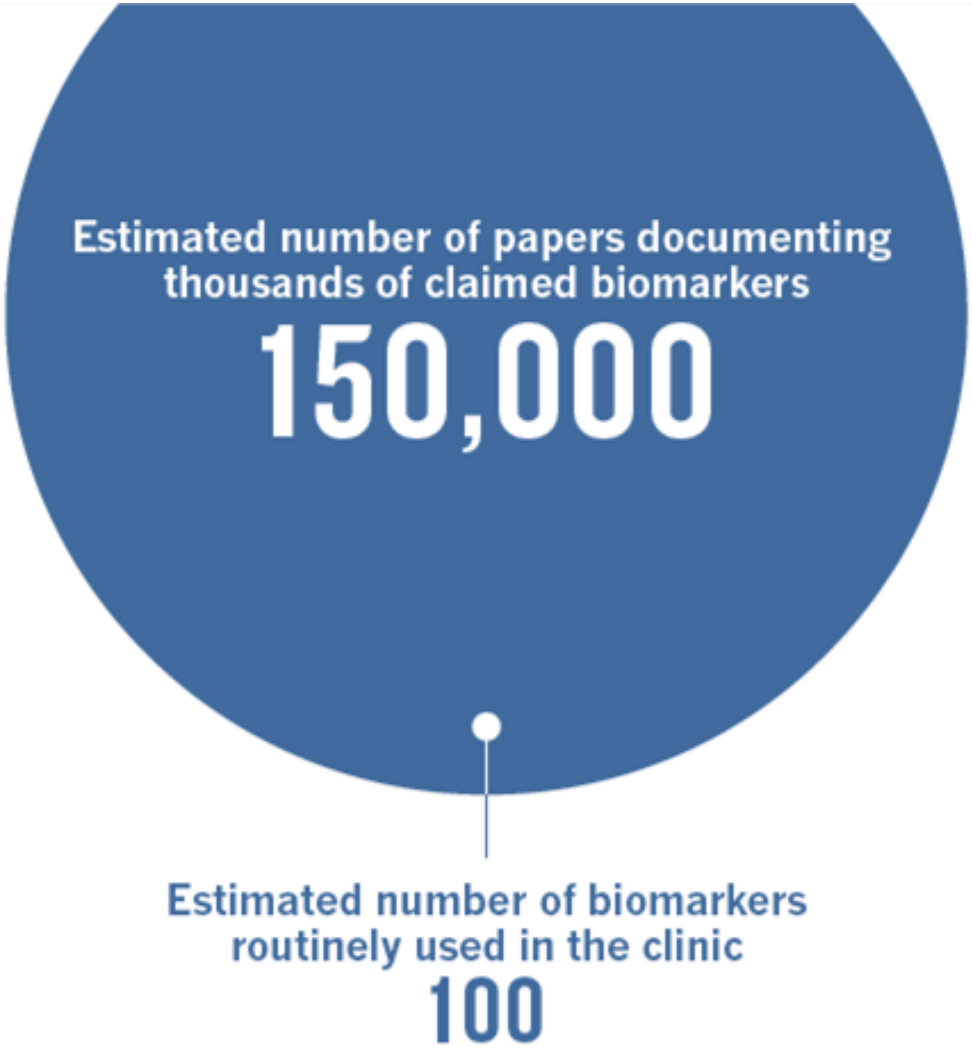
American Society of Clinical Oncology Perspective: Raising the Bar for Clinical Trials by Defining Clinically Meaningful Outcomes

Lee M. Ellis, David S. Bernstein, Emile E. Voest, Jordan D. Berlin, Daniel Sargent, Patricia Cortazar, Elizabeth Garrett-Mayer, Roy S. Herbst, Rogerio C. Lilenbaum, Camelia Sima, Alan P. Venook, Mithat Gonen, Richard L. Schilsky, Neal J. Meropol, and Lowell E. Schnipper

Thus, it is imperative that trial sponsors develop comprehensive biospecimen banks for each trial with informed consent from patients that will allow investigators to ask scientific questions before and after trials are completed to facilitate biomarker discovery and validation.[13]

Translational biomarker research – aims and challenges

(George Poste, Nature 2011: „dismal patchwork of fragmented research“)



Poste, Nature 2011

Level of evidence for biomarker studies

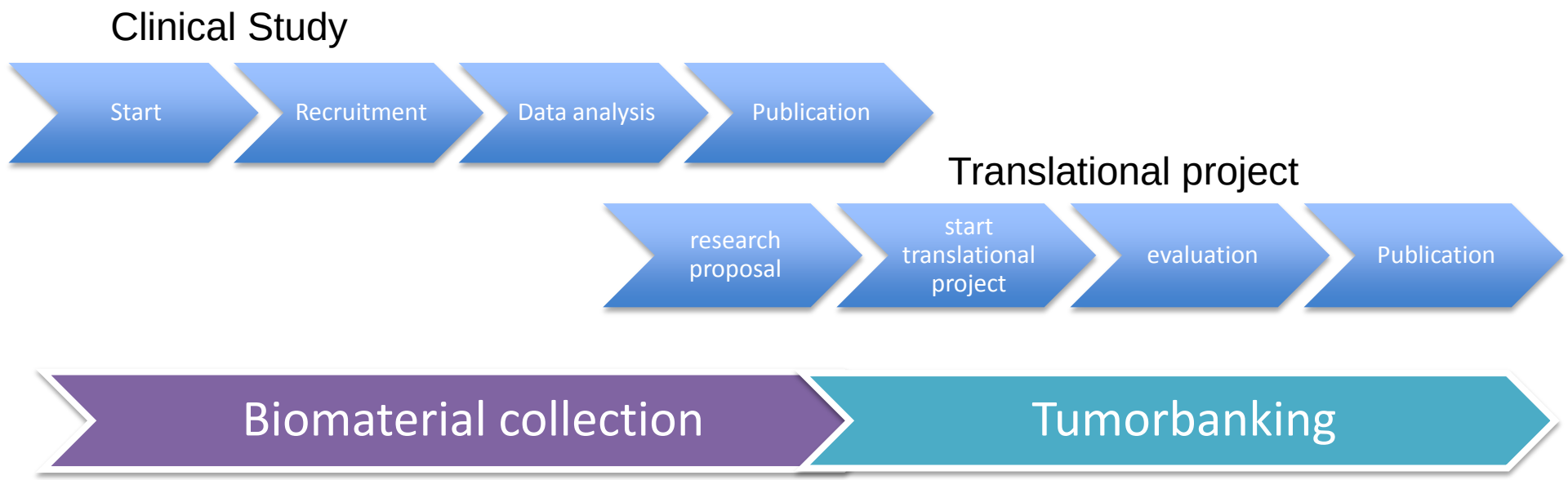
Type of tumor marker study		Definition	Possible level of evidence	
A	Prospective	clinical trial designed to address tumormarker	1	validation preferred, but not required
B	Prospective using archived samples "prospective-retrospective"	prospective biomarker design, existing samples collected in clinical trial	1	two studies with identical results
			2	only one study
C	Prospective observational	prospective registry and sample collection, no standardized treatment and follow-up	2	two studies with identical results
			3	only one study
D	Retrospective observational	collection of samples from archive, no standardized treatment	4-5	hypothesis generating, no clinical utility

Simon, Paik, Hayes JNCI, 2009

Requirements for a Marker-Based Test to Reach Level IB Evidence of Clinical Utility Based on Prospective-Retrospective Studies (McShane; Hayes, JCO 2012)

1. **"Adequate amounts of archived specimen** must be available from enough patients from a prospective trial ... for analyses to have adequate statistical power and for the patients included in the evaluation to be clearly representative of the patients in the trial.
2. The marker-based test should be **analytically and preanalytically validated** for use with archived specimens.
3. The **plan for marker evaluation** should be completely specified in writing before the performance of marker assays on archived specimens and should be focused on evaluation of a single completely defined markerbased test.
4. The **results from archived specimens should be validated** using specimens from one or more similar, but separate, studies."

Organisation of translational research



- There are many years between clinical study and translational research projects.
- Samples must be collected while the study is recruiting patients.
- Long-term storage is necessary.
- Research grant applications will only be successful of the samples have already been collected.

Legal requirements for tissue collection

(may be different in different countries)

- histological slides are part of the medical record and have to be stored for 10-15 years
- in certified breast cancer centers paraffin blocks have to be stored for 10-15 years
- certified centers are encouraged to participate in clinical studies, so the tissue might be transferred to study biobanks
- it is the patient's decision how the samples should be used
- pathologist might control if the patient has made an informed decision
- main risk: sample might be used completely in research and will not be available for further diagnostic

Informed consent issues regarding sample collections

(may be different in different countries)

- patients should give consent to central sample collection and long-term storage
- the right to use the sample is transferred to the biobank (ownership often remains with the patient)
- research purpose should be defined ("prognostic and predictive markers")
- it may be better not to mention specific markers
- patients should be informed that samples might be transferred to third parties and that results might be used commercially (e.g. patent applications)
- patient should be informed of the risk that the sample might be completely used in the research project and that a new biopsy might be required for future diagnostic tests

Sample collection and communication

Options for translational research

For pathologists

- Start research projects with clinical study groups
- everybody is looking for pathologists with research interest
- pathology techniques and expertise are needed
- Advantage: practice changing research is possible in connection with samples from clinical studies

For clinicians

- start communication with pathologists
- explain why sample collection is mandatory in clinical trials
- explain benefits for pathologists
- integrate pathologists in clinical study development
- include pathologists as PIs in translational research projects