Herlev og Gentofte Hospital

The Oncomine multi-dimensional biomarker analysis for future precision oncology: solid and blood malignancies

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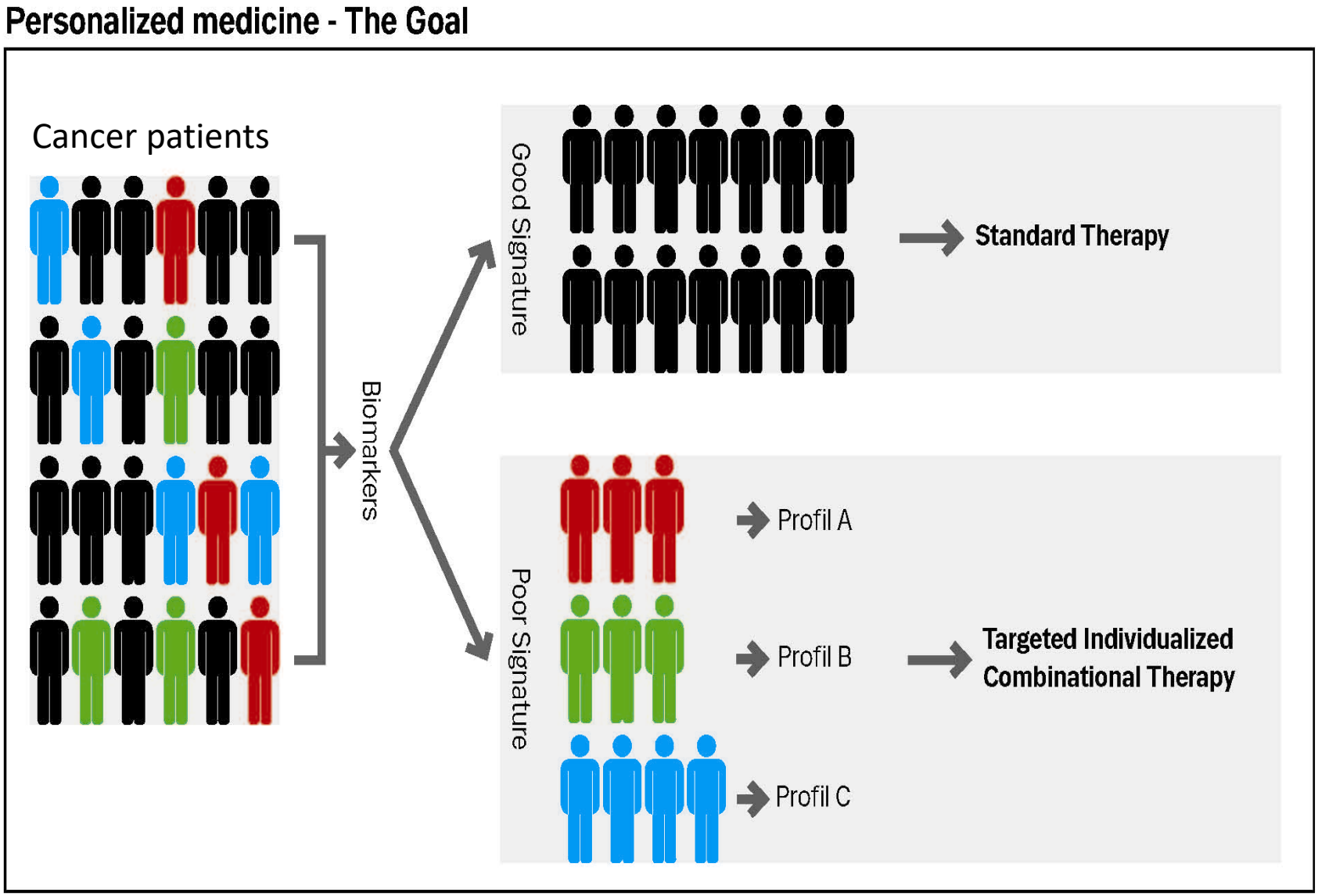
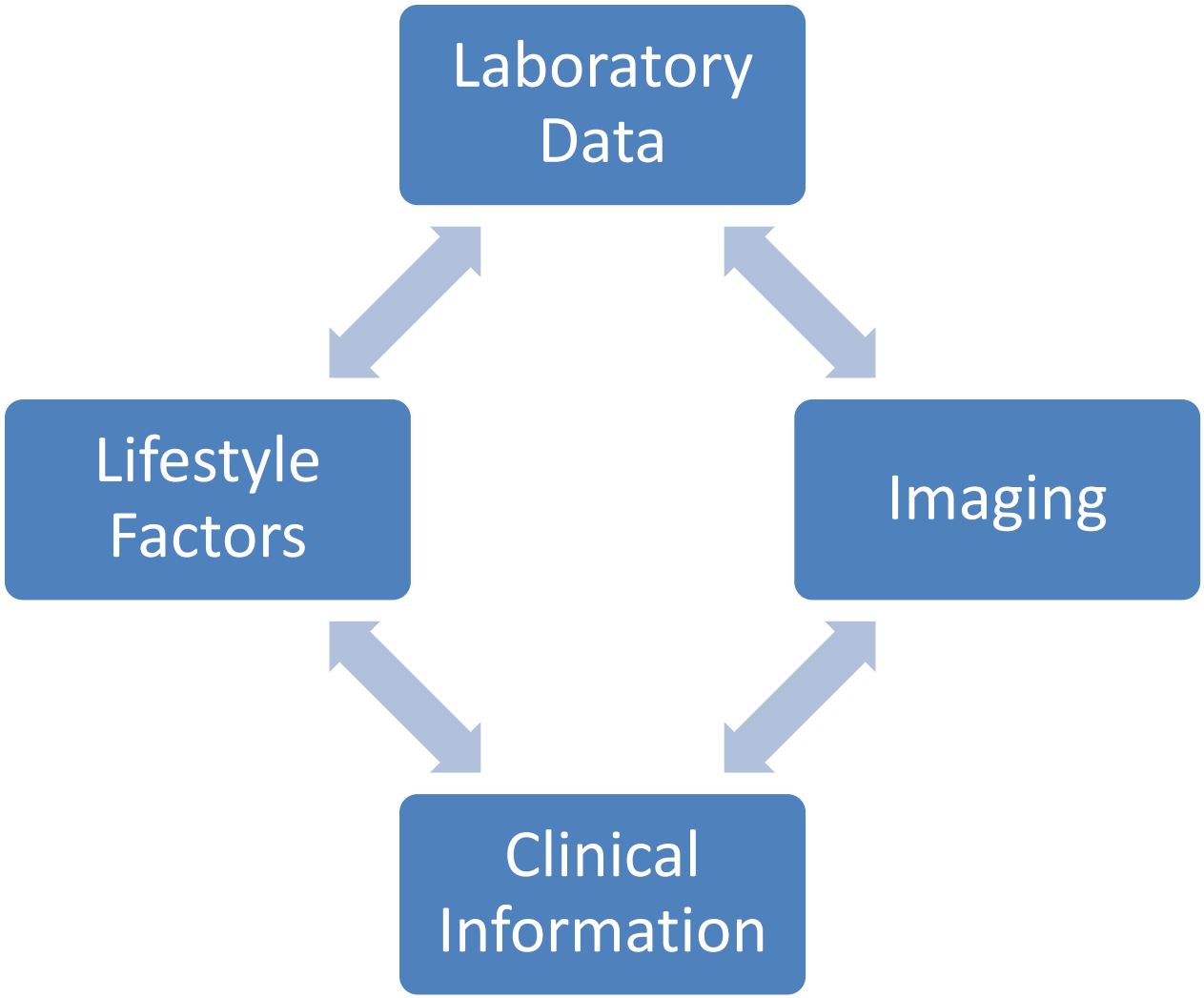
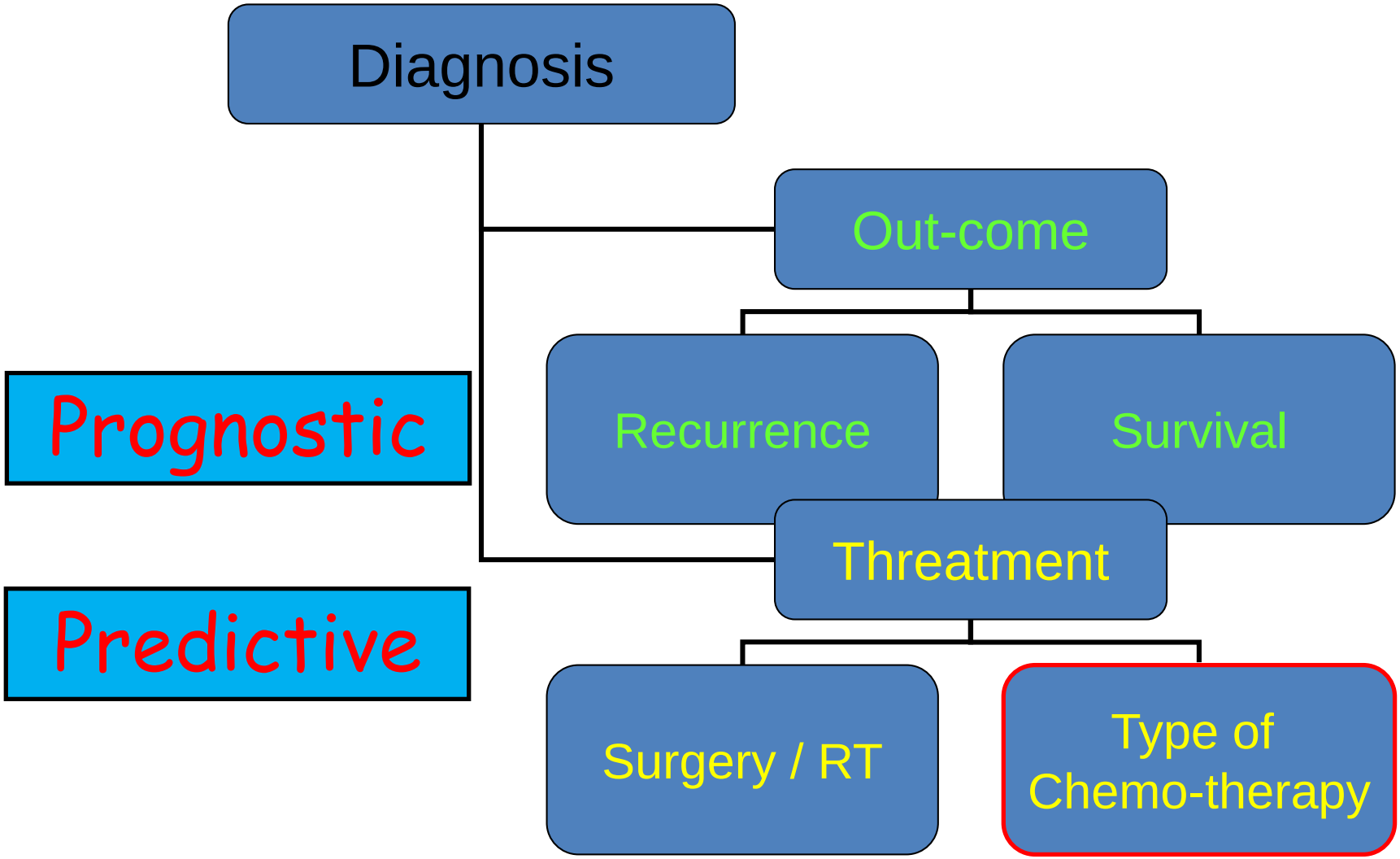
What should be obtained?



Do what is needed at the right time



Biomarkers may be used for



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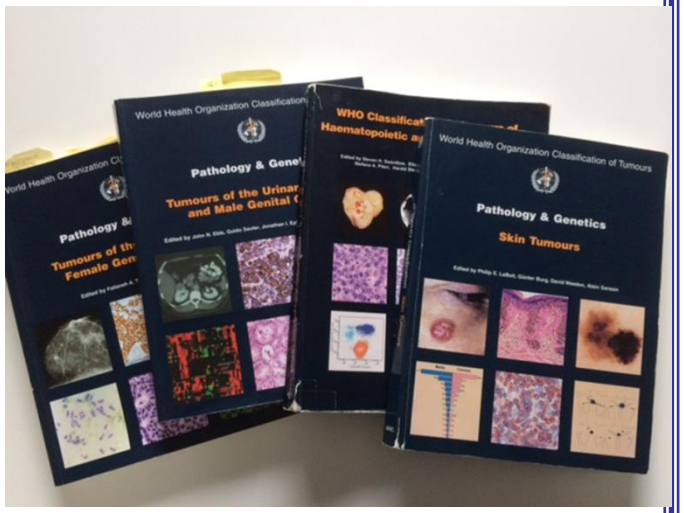
Denmark



Vision for our Pathology Department is: "Best possible pathology answer for the benefit of the patient“

Meaning that evidence-based results for current and future disease classifications (WHO) should be performed.

Laboratory analyses should support patient progress:

1. Diagnosis (Guideline - evidens) – selected biomarkers analyses:

Standard treatment/Trials

Predictive/Prognostic

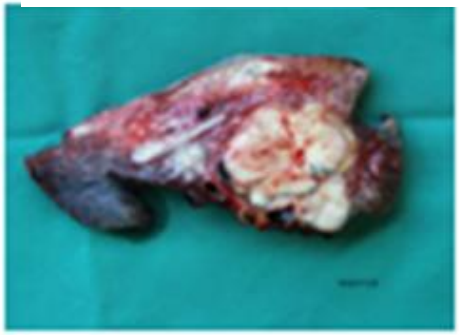
2. Experimental treatment – global biomarker analyses:

No evidens based treatment

Work at the pathology department

Surgery

Tissue



Pathology Dept.
Microscopy

Histopathology
(Light microscopy)

Protein expression
Immunohistochemistry

Genetic variations
Sequencing, array,
fragments analyses....

Diagnostic
Prognostic
Therapeutic
(Precision medicine)

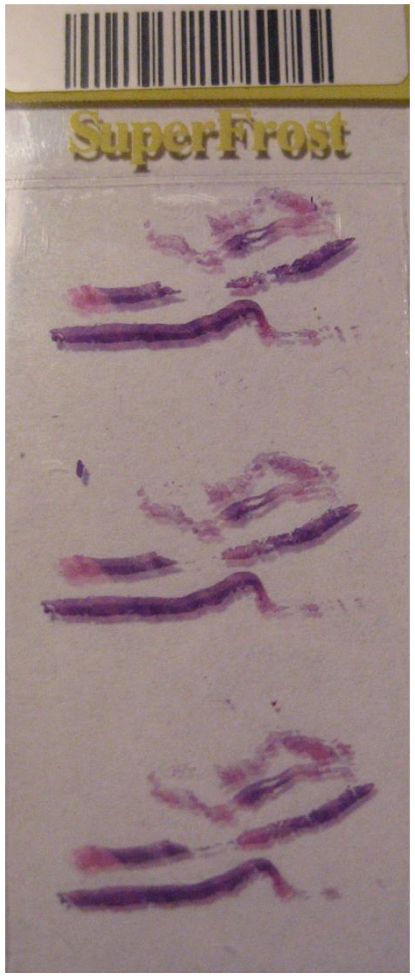
One combined Pathology answer

Diagnoses, coding, patobank,
Databases,
Danish CancerBiobank other
biobanks

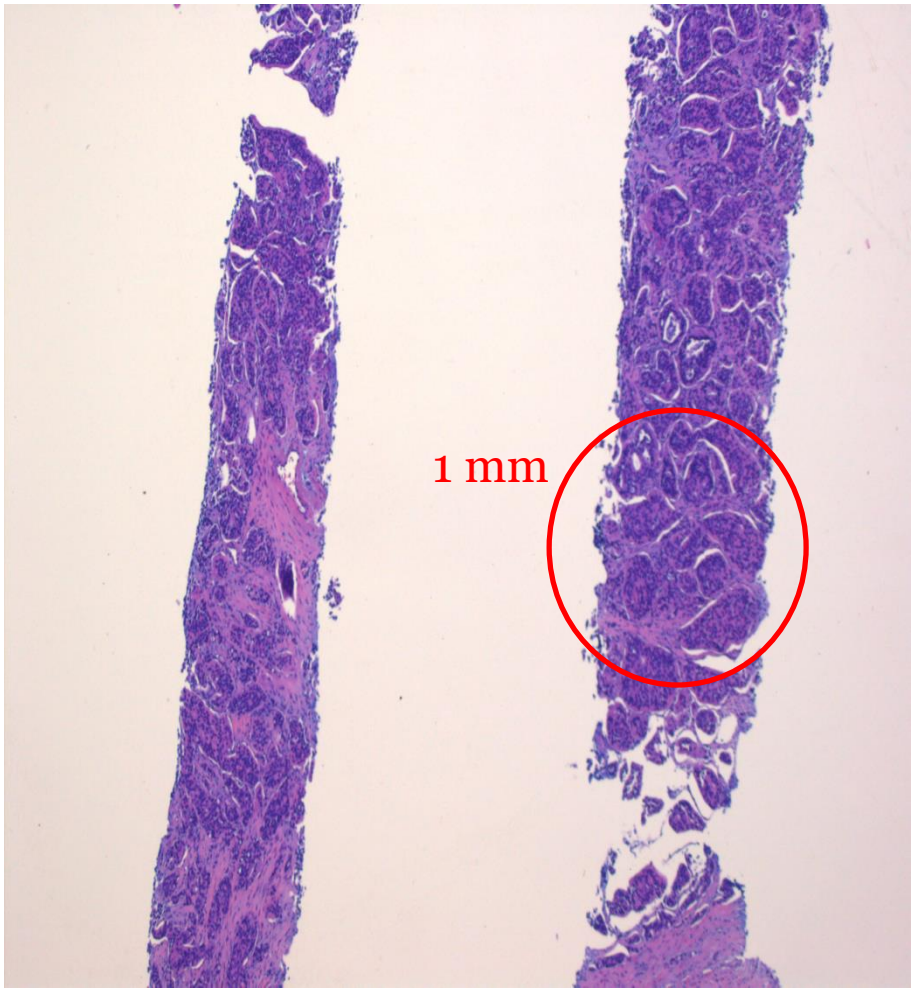
... **Personalized medicine**

Today: Material for molecular analyses at Pathology

Tissue slide



Stained needle biopsy – H&E stained



Future: Biobank material, crude tissue verified by pathologists – high quality DNA/RNA

Sequencing reactions in pathology for molecular carachterization

Samples with:

Colorectal cancer - KRAS, NRAS, BRAF, PIK3Ca (Cetuximab/ Panitumumab)

Gastrointestinal sarcoma tumors - c-KIT, PDGFa, KRAS, NRAS, BRAF, PIK3Ca (Imatinib)

Malignant melanoma – BRAF (Dabrafenib + Trametinib (MEK-inhibitor)

Lung cancer – EGFR (Erlotinib)

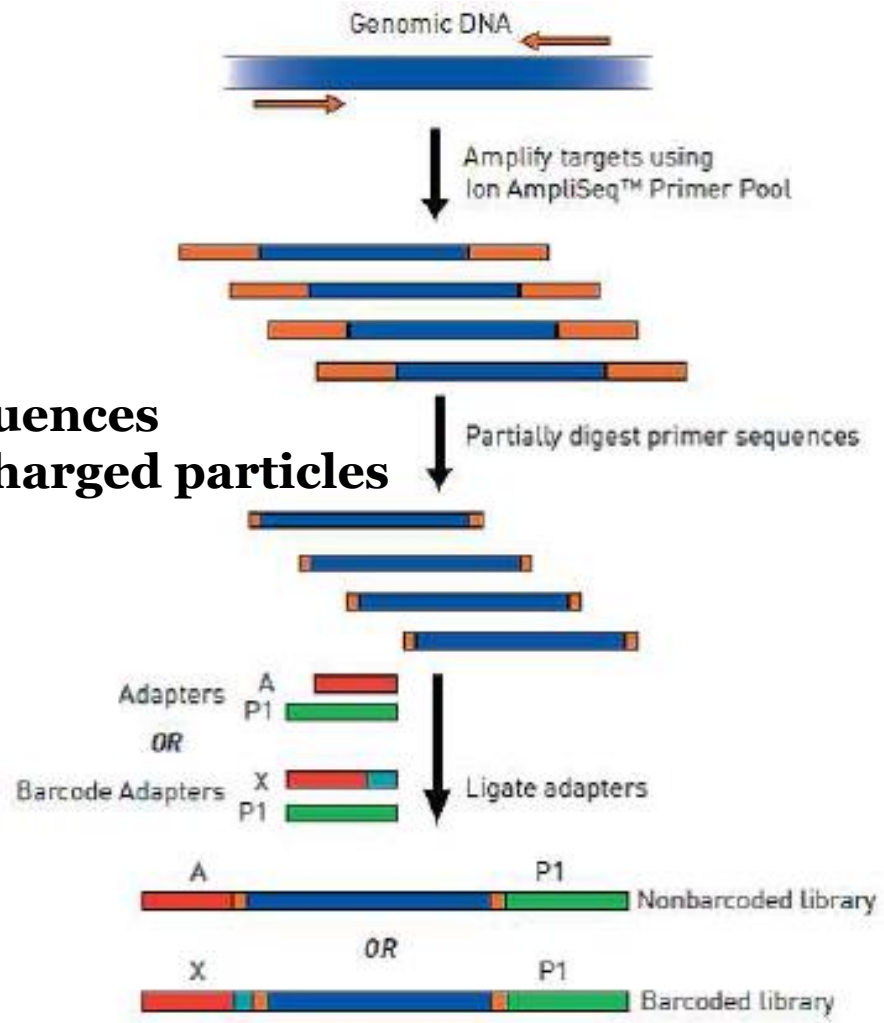
Ovarian cancer – BRCA1/2 (Olaparib)

More to come

for possible standardized evidence-based treatment

Library Preparation

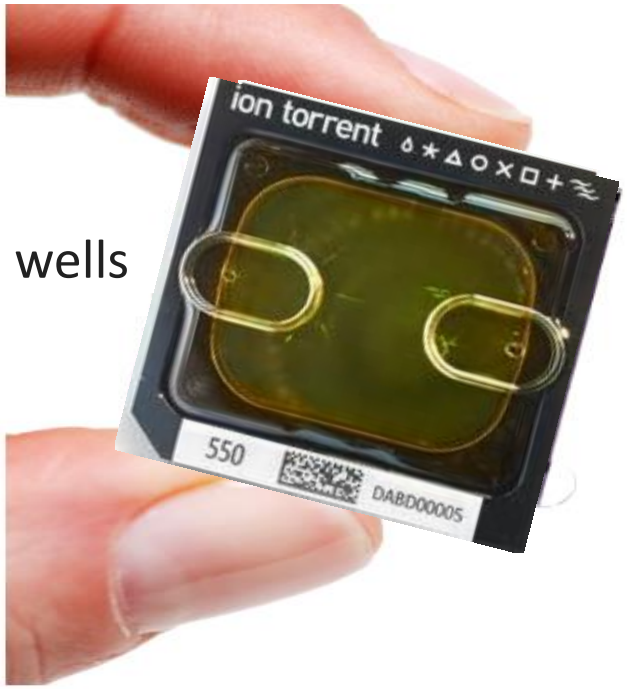
1. **Purification of DNA**
2. **Construction of library**
3. **Amplification of all sequences**
4. **Isolation of positively charged particles**
5. **Sequencing reaction**



Barcode

NGS

154 million wells



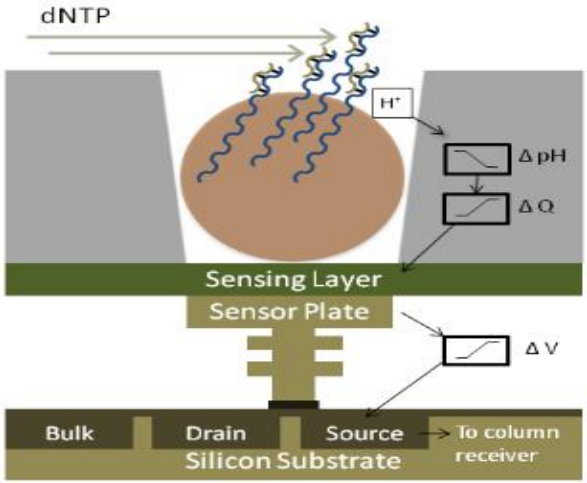
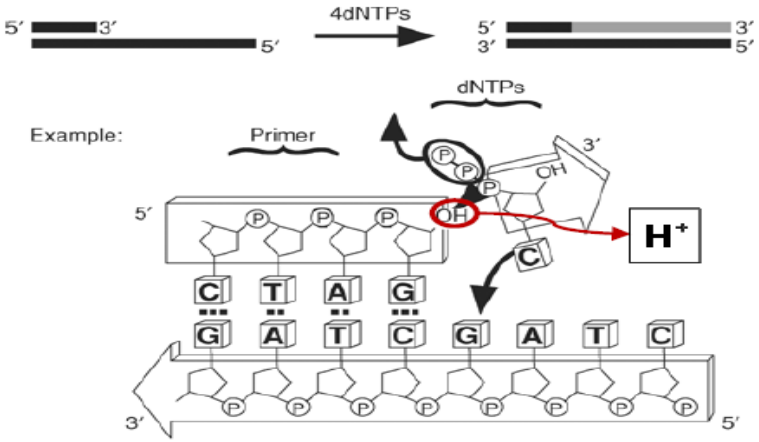


Figure 2. Schematic cross-section of a single well of an Ion Torrent sequencing chip. The well houses Ion Sphere™ particles containing DNA template. When a nucleotide incorporates, a proton releases and the pH of the well changes. A sensing layer detects the chage in pH and translates the chemical signal to a digital signal.



Chemical change (H+) are translated into a digital signal

Our throughput and the request for fast answers enhanced and we therefore perform NGS (panels and exome) twice a week on larger chips

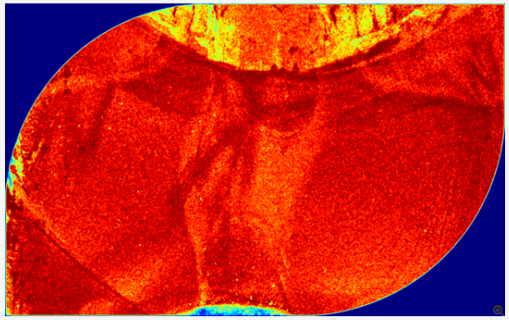
Results

414 M
Total Bases

94
Key Signal

89 %
ISP Loading

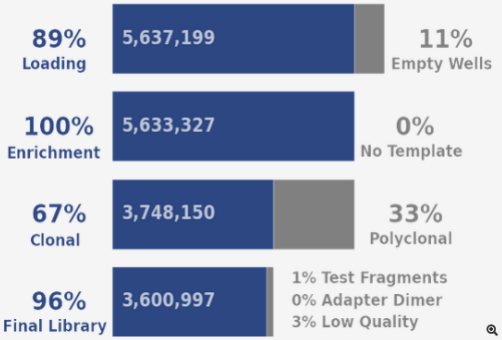
ISP Density



3,600,997
Total Reads

64 %
Usable Reads

ISP Summary

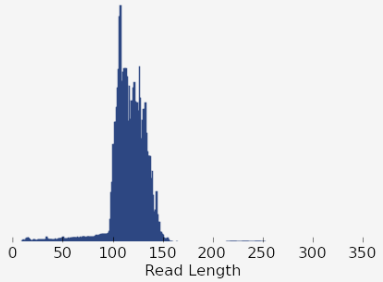


115 bp
Mean

116 bp
Median

107 bp
Mode

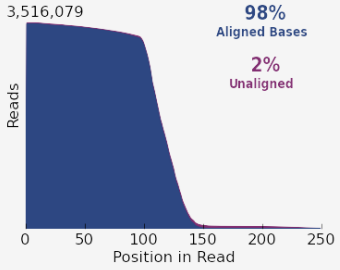
Read Length



Aligned to Homo sapiens

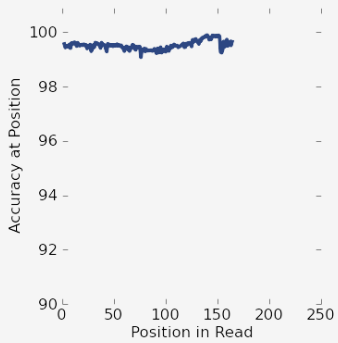
396 M
Total Aligned Bases

0.1X
Reference Coverage



	Count	%
Total Reads	3,516,079	—
Aligned Reads	3,499,047	99.5%
Unaligned Reads	17,032	0.5%

99.5%
Mean Raw Accuracy 1x



382 M
AQ17 Total Bases

Alignment Quality

	AQ17	AQ20	Perfect
Total Number of Bases [bp]	382 M	364 M	321 M
Mean Length [bp]	112	109	99
Longest Alignment [bp]	281	281	281
Mean Coverage Depth [x]	0.1	0.1	0.1

Results

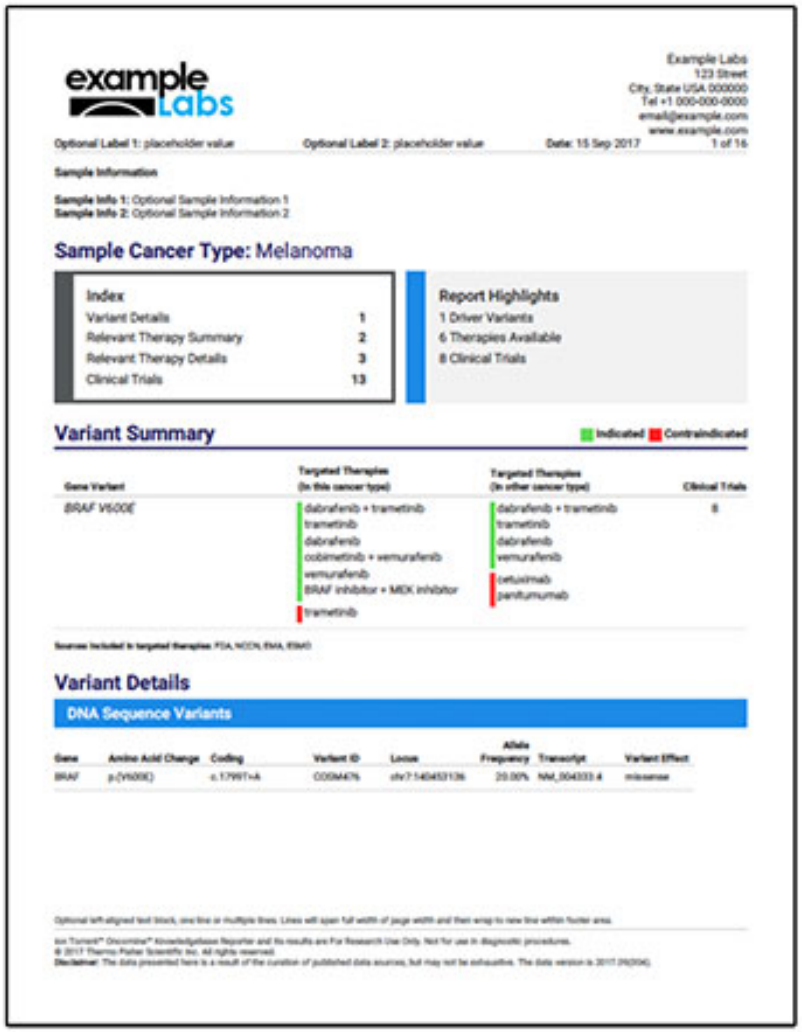
Future:Automatization

Molecular biologists are doing all lab work and datamining

REGION

Herlev Hospital

Oncomine™ knowledgebase reports are made as basis for results evaluation in Pathology



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www.example.com

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Sample Information

Sample Info 1: Optional Sample Information 1
Sample Info 2: Optional Sample Information 2

Sample Cancer Type: Melanoma

Index	
Variant Details	1
Relevant Therapy Summary	2
Relevant Therapy Details	3
Clinical Trials	13

Report Highlights
1 Driver Variants
6 Therapies Available
8 Clinical Trials

Variant Summary

Indicated Contraindicated

Gene Variant	Targeted Therapies (In this cancer type)	Targeted Therapies (In other cancer type)	Clinical Trials
BRAF V600E	dabrafenib + trametinib trametinib dabrafenib cobimetinib + vemurafenib vemurafenib BRAF inhibitor + MEK inhibitor trametinib	dabrafenib + trametinib trametinib dabrafenib vemurafenib cetuximab panitumumab	8

Sources included in targeted therapies: FDA, NCCN, EMA, ESMO

Variant Details

DNA Sequence Variants

Gene	Amino Acid Change	Coding	Variant ID	Locus	Allele Frequency	Transcript	Variant Effect
BRAF	p.(V600E)	c.1799T>A	COSM476	chr7:140453136	20.00%	NM_004333.4	missense

Optional left-aligned text block, one line or multiple lines. Lines will span full width of page width and then wrap to new line within footer area.

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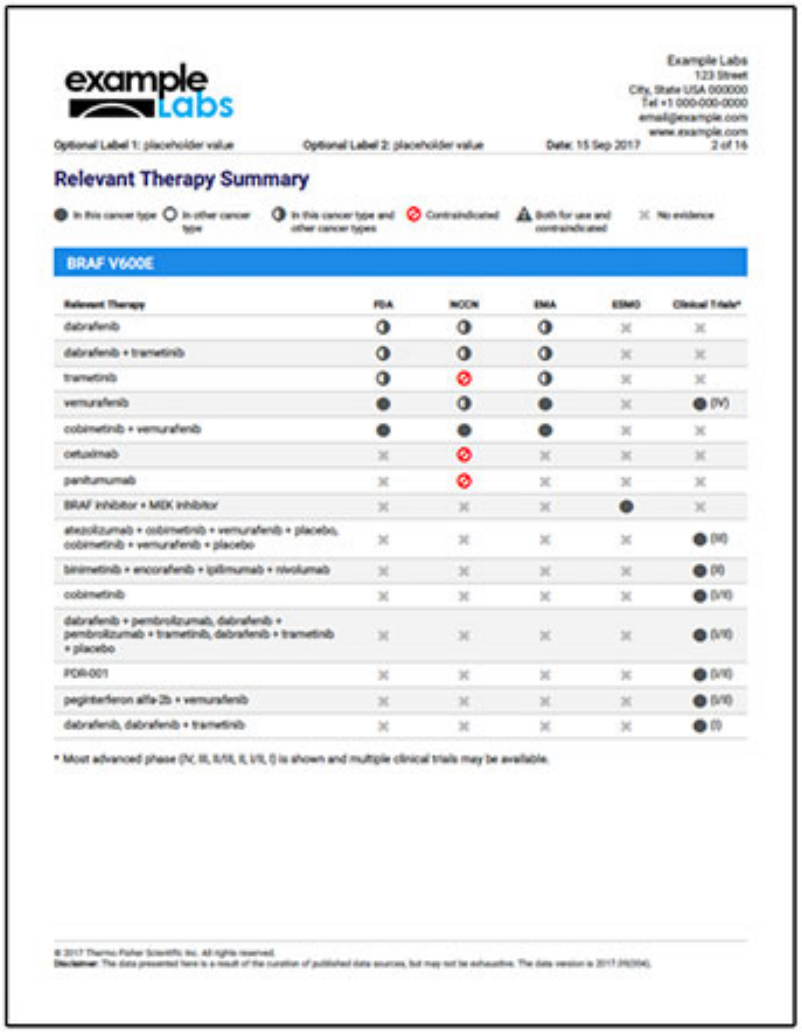

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Relevant Therapy Summary

● In this cancer type ○ In other cancer type ◑ In this cancer type and other cancer types ⊘ Contraindicated ⚠ Both for use and contraindicated ✕ No evidence

BRAF V600E

Relevant Therapy	FDA	NCCN	EMA	ESMO	Clinical Trials*
dabrafenib	◑	◑	◑	✕	✕
dabrafenib + trametinib	◑	◑	◑	✕	✕
trametinib	◑	⊘	◑	✕	✕
vemurafenib	●	◑	●	✕	● (IV)
cobimetinib + vemurafenib	●	●	●	✕	✕
cetuximab	✕	⊘	✕	✕	✕
panitumumab	✕	⊘	✕	✕	✕
BRAF inhibitor + MEK inhibitor	✕	✕	✕	●	✕
atezolizumab + cobimetinib + vemurafenib + placebo, cobimetinib + vemurafenib + placebo	✕	✕	✕	✕	● (III)
binimetinib + encorafenib + ipilimumab + nivolumab	✕	✕	✕	✕	● (II)
cobimetinib	✕	✕	✕	✕	● (I/II)
dabrafenib + pembrolizumab, dabrafenib + pembrolizumab + trametinib, dabrafenib + trametinib + placebo	✕	✕	✕	✕	● (I/II)
PDR-001	✕	✕	✕	✕	● (I/II)
peginterferon alfa-2b + vemurafenib	✕	✕	✕	✕	● (I/II)
dabrafenib, dabrafenib + trametinib	✕	✕	✕	✕	● (I)

* Most advanced phase (IV, III, II/III, II, I/II, I) is shown and multiple clinical trials may be available.

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Patologiafdelingen
Herlev og Gentofte Hospital

Molecular results are included in the final report

Herlev Hospital
Patologiafdelingen
Tlf.: 4488 3326

Rekv.nr

Special /
KOPI IKKE GODKENDT SVAR

Herlev Hospital
Herlev hospital Patologi afdeling
Ringvejen 75
2730 Herlev

Rekv.læge:
Rekvireret: 24.03.2015
Modtaget: 24.03.2015 10:30

Patient: **Testpers**

Materiale:
01: BRAF på blok:

Diagnoser:
01: Blod
-
polyarteritis
BRAF genmutation V600E
KRAS genstatus normal
NRAS genstatus normal
PIK3CA genændring uden kendt behandlingskonsekvens
polymerase kædereaktion (PCR) analyse

Flowcytometri
01: ggg

PCR-undersøgelse
01: KRXXXX - 15hehXXXXXX
Der er Fundet BRAF mutation (c.1799T>A, p.V600E)
Der er ikke fundet KRAS eller NRAS mutation, altså har patienten vildtype KRAS og NRAS gener i det undersøgte materiale.
Mutationer med ukendt behandlings indikation:
PIK3CA mutation (c.1173A>G, p.I391M) I exon 7.
Mutationsanalysen er foretaget med Next Generation Sequencing (NGS) af KRAS genet (codon 1-189), NRAS genet (codon 1-189), BRAF genet (codon 1-664 og 669-766), PIK3CA genet (codon 1-1068), AKT1 genet (1-131, 146-384, 392-480) samt PTEN (1-267 og 271-403), disse sekventeres og rapporteres. Da behandlingskonsekvensen ikke kendes for generne TP53, ARID1A, TGFBR2, MLH1, CTNNB1, SMAD2, MSH2, MSH6, EGFR FAM123B, ATM, APC, MET, FBXW7 og CASP8 rapporters disse ikke. Ved behov for databehandling og rapportering af disse gener kan molekylærenheden Herlev patologiafdeling kontaktes.
Databehandling af individuel genetisk variation besvares på baggrund af ratio, samt varians coverage mod reference coverage.
Bemærkning: LabID: KR1222, polymorfismer angives ikke.

Udskrevet den 21.03.2015 kl. 09:26 Side 1 Mere ...

Also included are version of panel, software version etc.

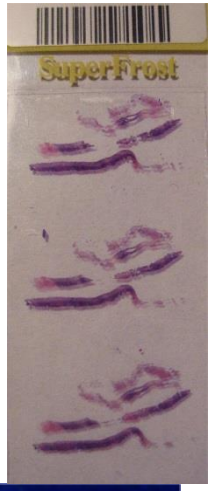
Samples tested

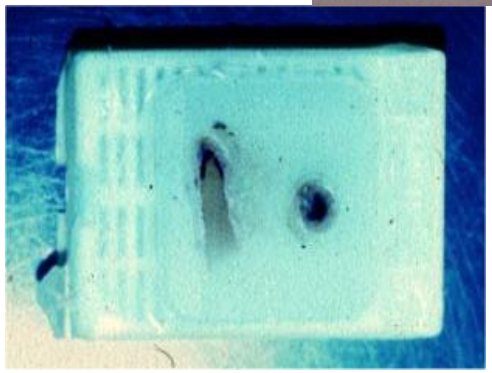
Solid tumors:

Colorectal cancer approx. 750 samples
GIST approx. 100 samples
Malignant melanoma approx. 50 samples
Ovarian
Breast } **100 patients**
Prostate

Haematology (blood):

MDS/AML approx. 200 samples
STAT3 approx. 10 samples
TP53 approx. 100 samples
c-KIT approx. 30 samples





Panels in use

DNA based:

7 genes (7 genes, hot spot - panel)

11 genes (7 gener +extra, all coding areas) – the panel we initially used

22 genes (hot spot – literature based)

Oncomine focus assay (52 genes – routine – literature/experience)

11 genes GIST (all coding areas) combined with Sanger Sequencing

BRCA1/2 (germline) alternatively oncomine comprehensive v3 (somati

AML, MDS_MPN custom design/ commercial

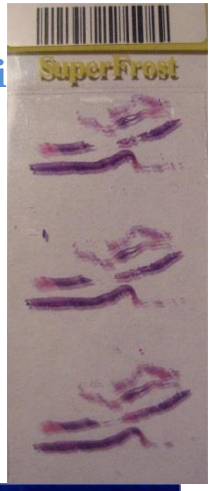
cKIT custom designed

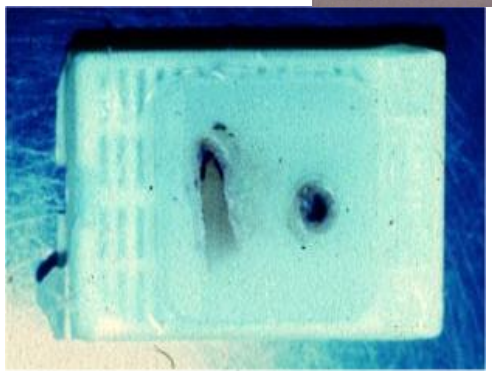
STAT3 custom designed

TP53 custom designed

RNA based:

Oncomine™ Focus Assay (fusions – NTRK, FGFR2)





Present analysis flow

Colorectal cancer:

Oncomine™ Focus Assay (DNA) +MSI

to consider: MSI high+BRAF WT then Oncomine Focus or Archer (RNA): fusion - NTRK

GIST:

NGS11G (all coding sequence) + Sanger:

c-KIT, PDGFRA, NF1, BRAF, KRAS, NRAS, PIK3CA, PTEN, SDHB, SDHC, SDHD

If WT in c-KIT, PDGFRA then Oncomine™ Focus Assay (RNA): fusion - NTRK

Malignant melanoma:

Oncomine™ Focus Asay (BRAF)

Ovarian:

BRCA1/2 (all coding sequence) + Oncomine™ Comprehensive + HRR*

to consider HRD (HRR+ WGA)

Breast:

BRCA1/2 (all coding sequence) + Oncomine™ Comprehensive

to consider HRD (HRR+ WGA)

Prostate:

BRCA1/2 (all coding sequence) + Oncomine™ Comprehensive

*HRR: ATM, BRAD1, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D og RAD54L

Up-comming analysis flow

Clonality, hyper mutation:

IGHV-PGM – demand high quality DNA. No commercial software

Lymphome:

Costumer designed panel

Breast cancer:

PIK3Ca (SOLE study)

SOLE:
About 40% of samples with HR+ breast cancer have PIK3CA mutations, activating the PI3 kinase pathway leading to cancer progression and resistance to endocrine therapy. Alpelisib (BYL719) is an oral PI3K inhibitor that is alpha specific. “The alpha isoform of PI3-kinase is the one that is mutated in breast cancer.

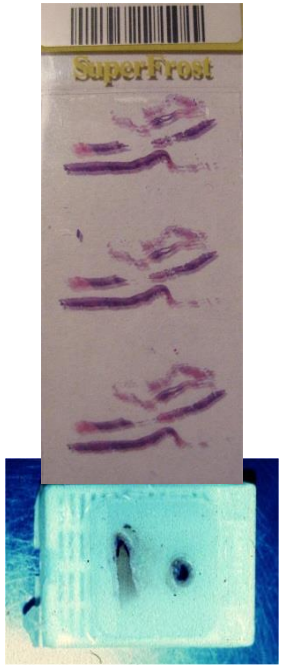
Cholangio carcinoma:

HER2 and BRCA1/2

Oncomine™ Focus Assay (RNA) or Archer: fusion – FGFR2 – validation trial

Pancreatic cancer:

HER2, MSI and BRCA ½ +HRR (POLE study)

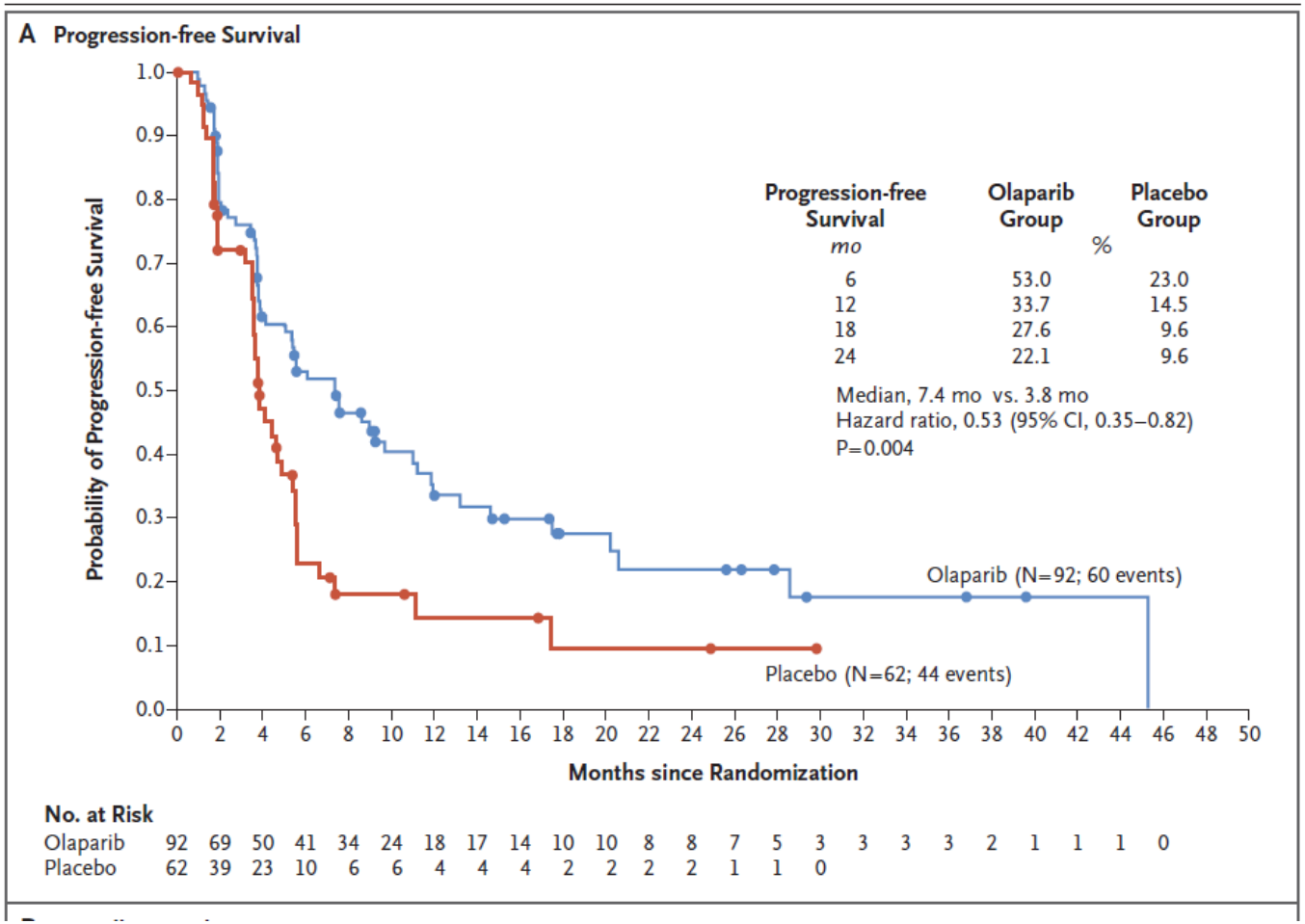


History

Earlier studies of ovarian cancer have shown that germline and somatic BRCA mutations have benefit of treatment with inhibitor of the enzyme poly (ADP-ribose) polymerase (PARP), an repair enzyme of DNA single strenght DNA damage.

The PARP-inhibitor Olaparib (Lynparza) was in 2014 aproved due to a randomized phase II trial. This study showed that treatment with Lynparza (400 mg x 2 dgl) after response of platin-based chemotherapy treatment significantly prolong the progression-free survival of patients with BRCA mutations
(median PFS 11,2 vs. 4,3 mths, P < .0001), [Ledermann et al, Lancet oncol. 2014]

At ASCO 2019 the POLO study presented their results indicating a prolonged survival of 3 mths for patients with pancreatic cancer habouring a germline BRCA1/2 mutation (median PFS 7.4 vs. 3.8 mths).



Article POLO:
In conclusion, the POLO trial showed that maintenance olaparib provided a significant progression-free survival benefit to patients with a germline *BRCA* mutation and metastatic pancreatic cancer that had not progressed during platinum-based chemotherapy.

Experimental treatment Clinical Phase I unit

When there are no more standard treatments

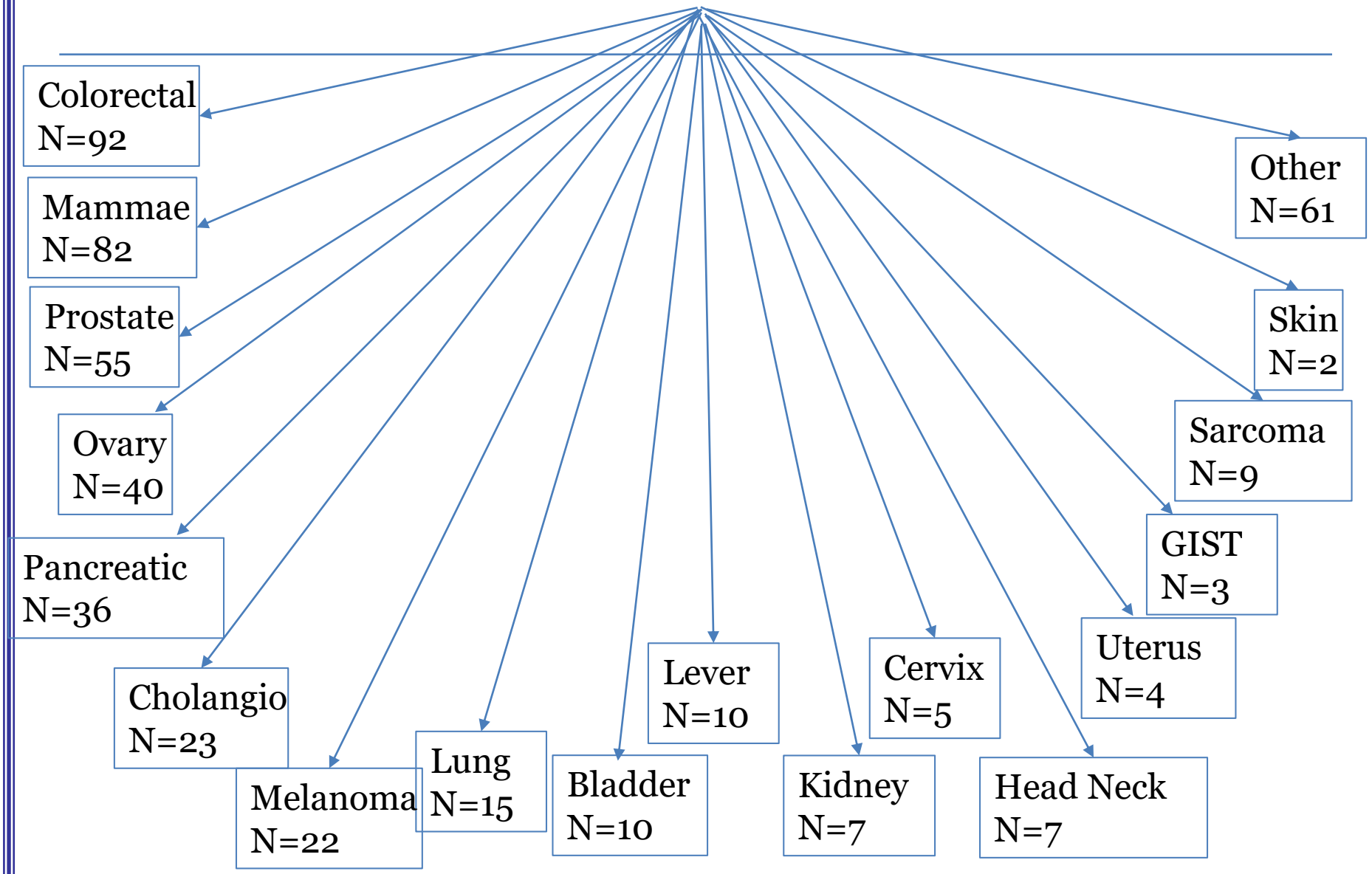
Weekly multidisplinary board meetings is established with the participation of all specialists

Experimental treatment Clinical Phase I unit

When there are no more standard treatments

From 2015 a total of approx. 4500 samples have been tested in the unit

Total number of samples being discussed based on sequencing (N=483)



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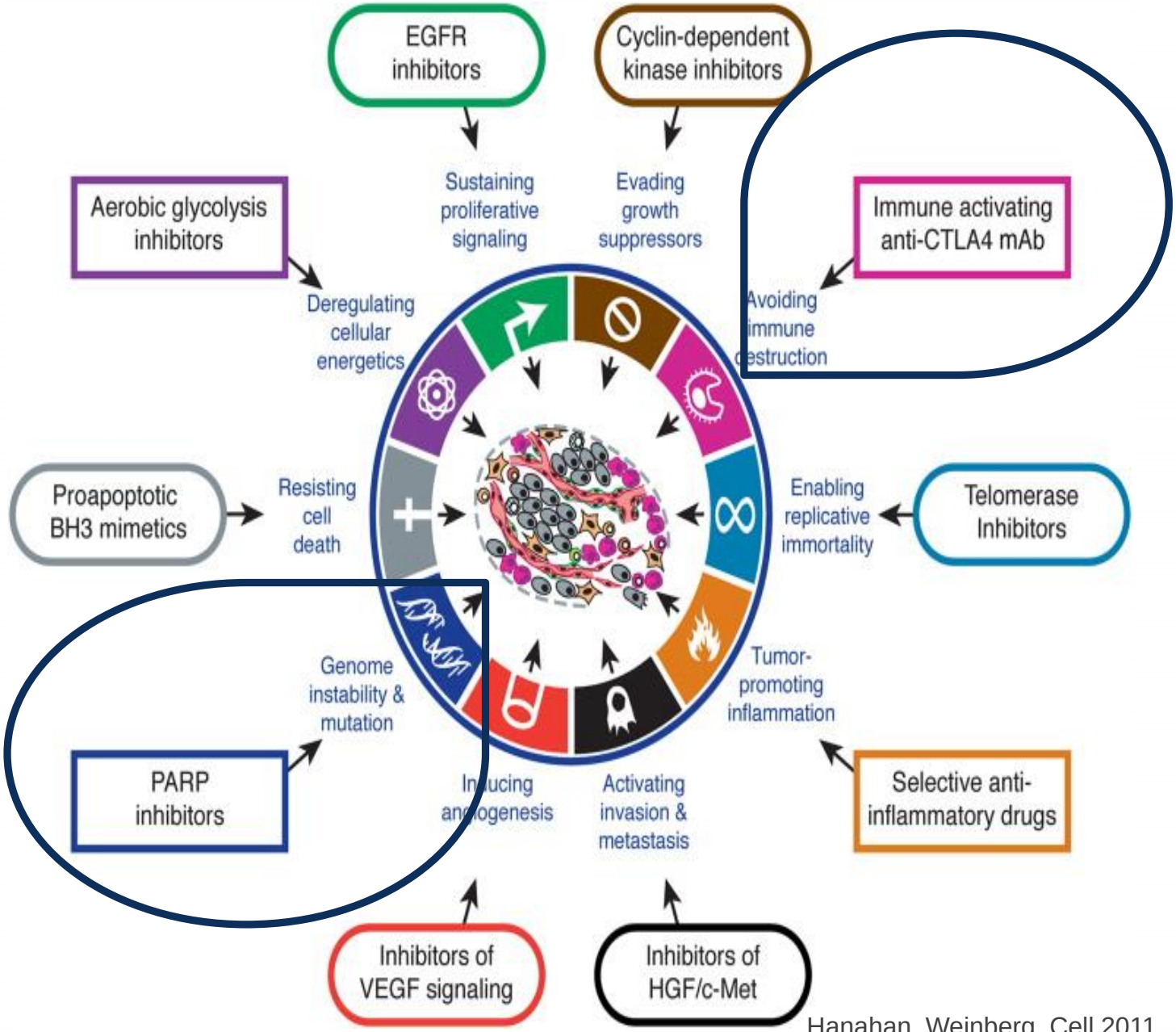
HALLMARKS OF CANCER AND TARGETED THERAPY



Hanahan, Weinberg, Cell 2011

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Targeted individualised cancer therapy – What is it?

Targeted therapy: Molecules that interfere with a cancer cell growth

In the last two decades there has been two main fields of cancer research - and targeted therapy focus on these cellular changes:

1. **Genetic driver mutations**
2. **Tumor immunity**

Both fields have resulted in a number of targeted therapies for a multiple of different cancer forms.

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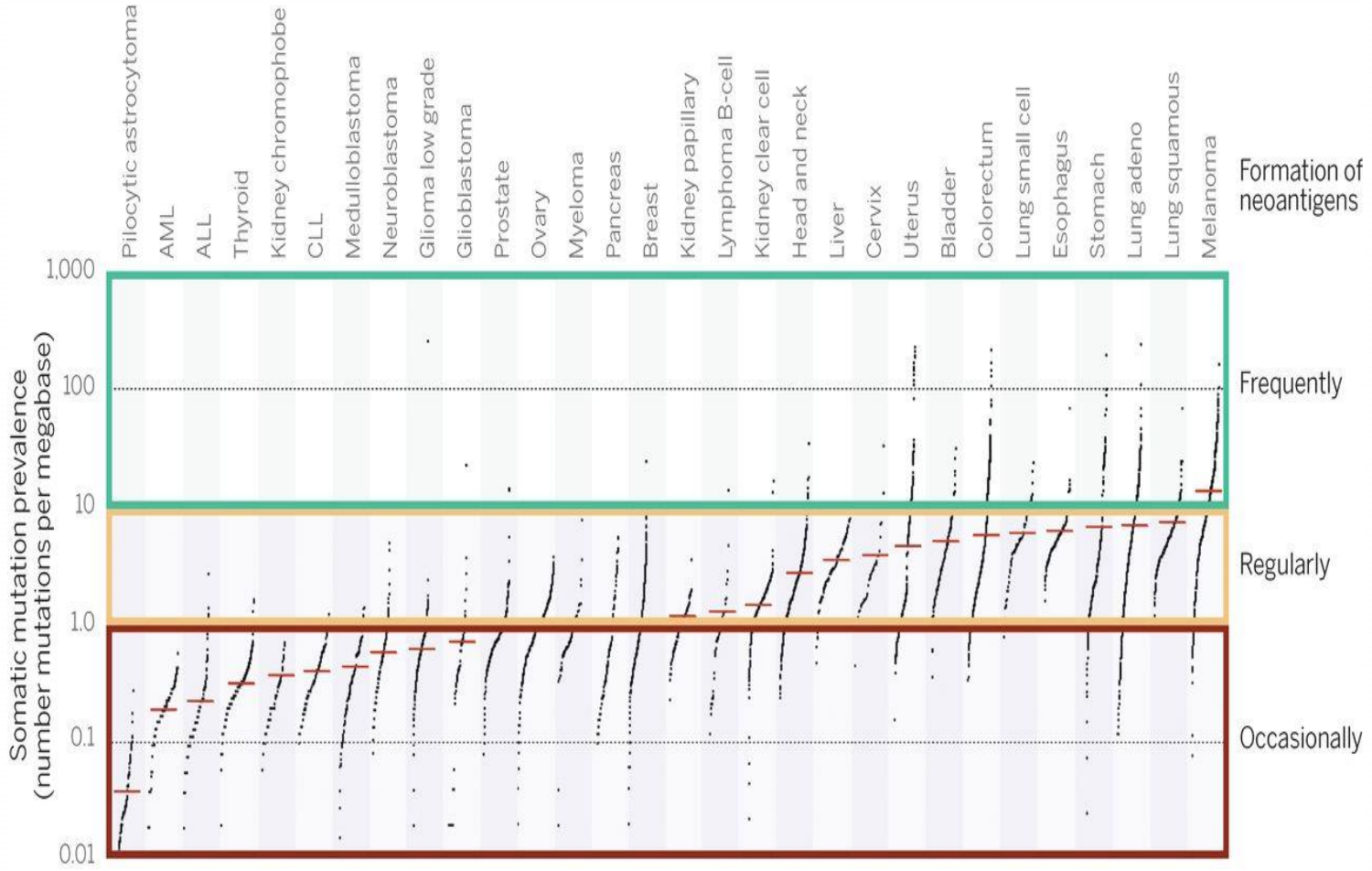
Tumor immunity: Neo-antigens



Schumacher et al, Science 2015

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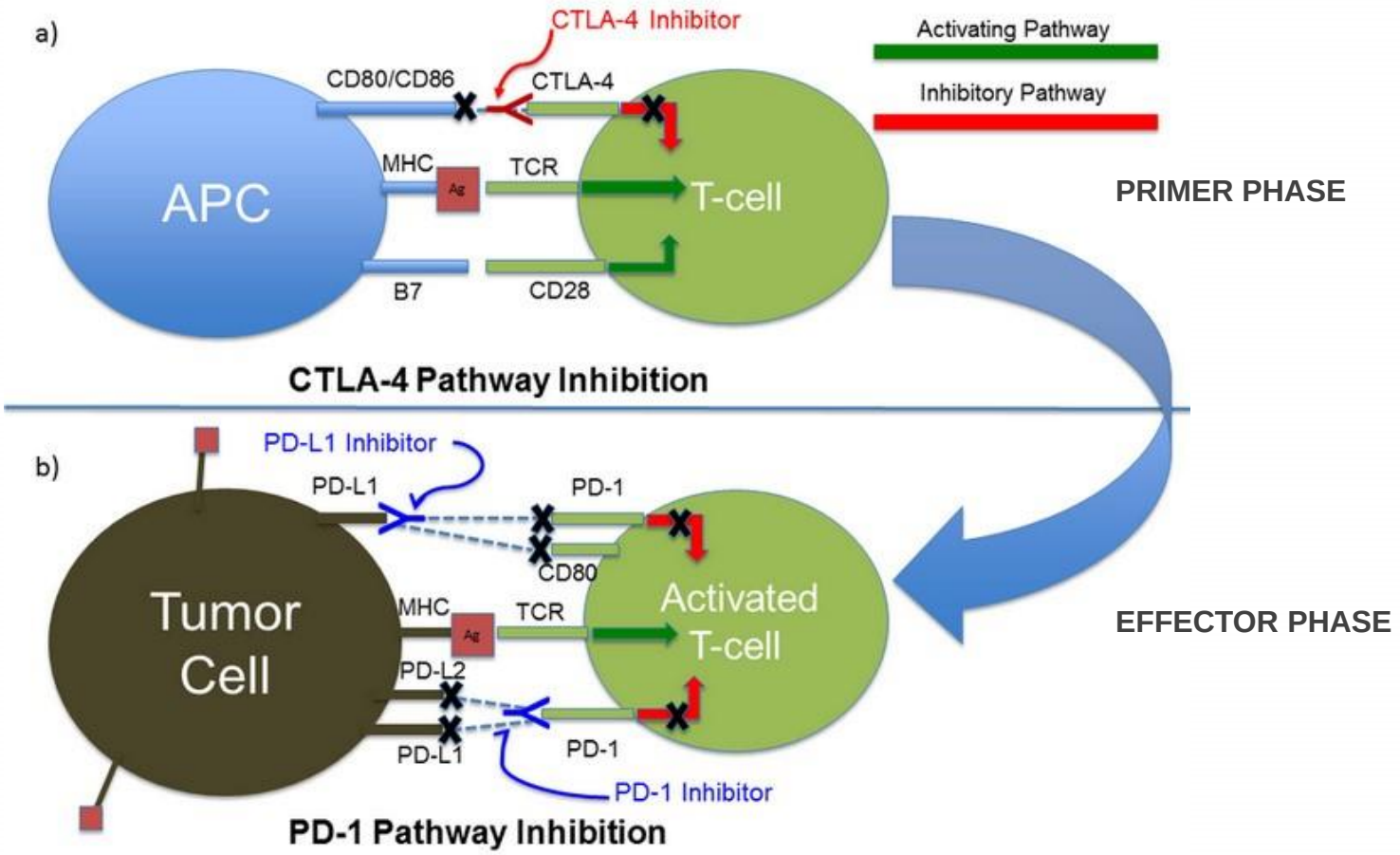
Check point inhibitors **are a type of immunotherapy**

Work by blocking proteins that stop immune system from attacking the cancer cells



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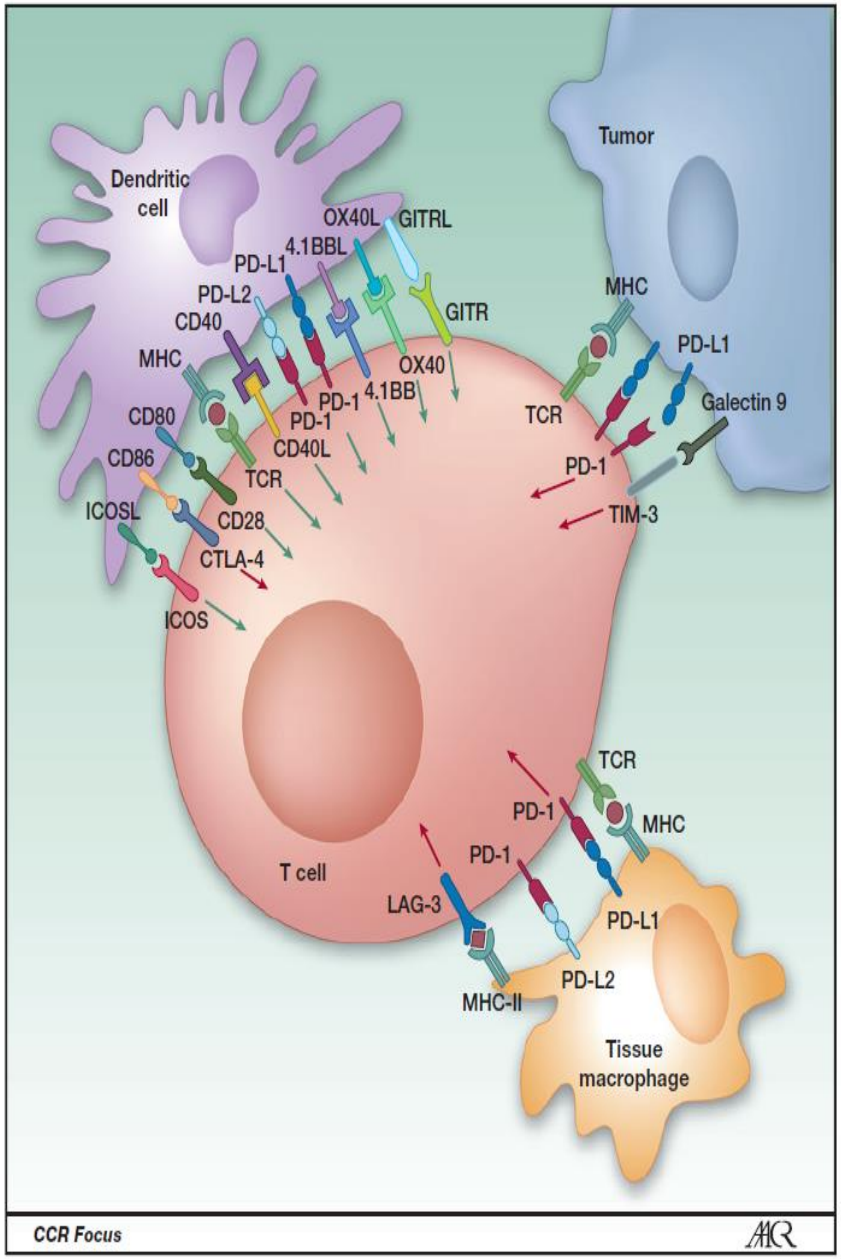
Immunotherapy

1. Check point inhibitors:
   - **CTLA-4 inhibitor**: Ipilimumab
   - **PD-1 inhibitor**: Nivolumab
   - **PD-L1 inhibitor**: Pembrolizumab, Atezolizumab, Avelumab
2. Vaccines
3. T cell therapy



Ott PA et al, Clin Can Res 2013

Panels used in Pathology

Experimentel treatment:

All tumor types – approx. 500 patients

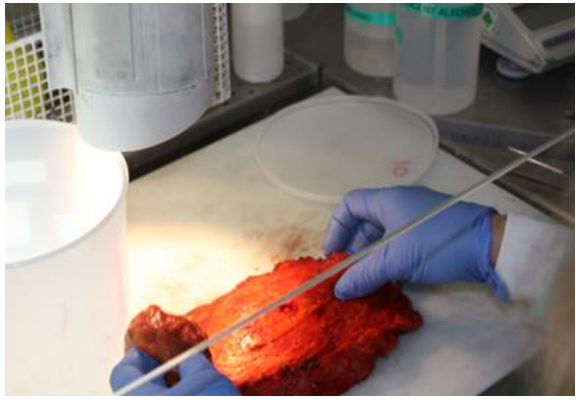
143 genes (Oncomine™ Comprehensive panel)

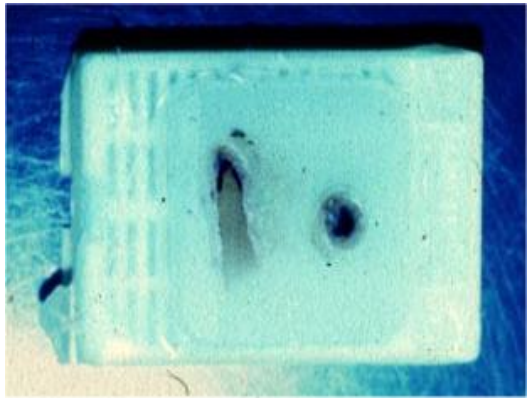
409 genes - Ion amplification Immune Repertoire Assay, Plus,

403 genes - AmpliSeq™ Comprehencive Cancer panel

407 genes – Oncomine™ Tumor mutation load Assay

Exome sequencing





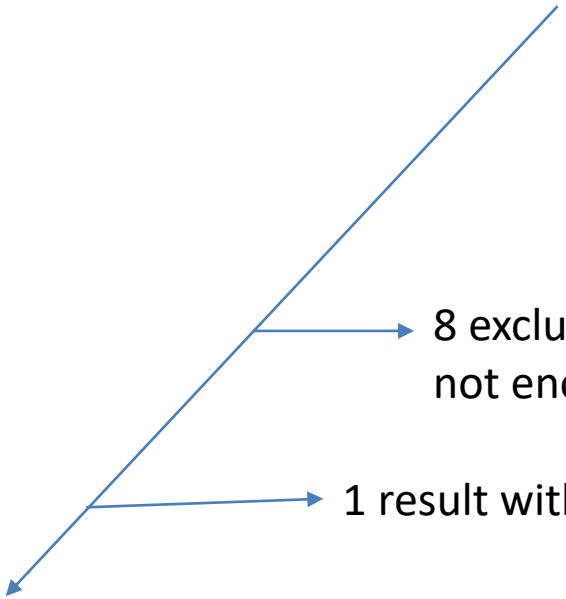
Tumor Mutational Burden

- Genomic biomarker measurering changes in DNA (no. mutations pr. megabase DNA)
- High score (high no of mutations) is used to predict response of treatment with checkpoint inhibitors (immunotherapi)
- High no of mutations is associated with high production of neoantigens, recognized by immune system, shown in previous figure
- Cut-off, what is the correct cut-off? Same for all tumor types? Same for blood and tissue?

We do not know

Comparison study FMI/Thermo

45 test/samples – all selected from samples being through fase I unit. All samples being sequenced by large panel. All samples being sparce.

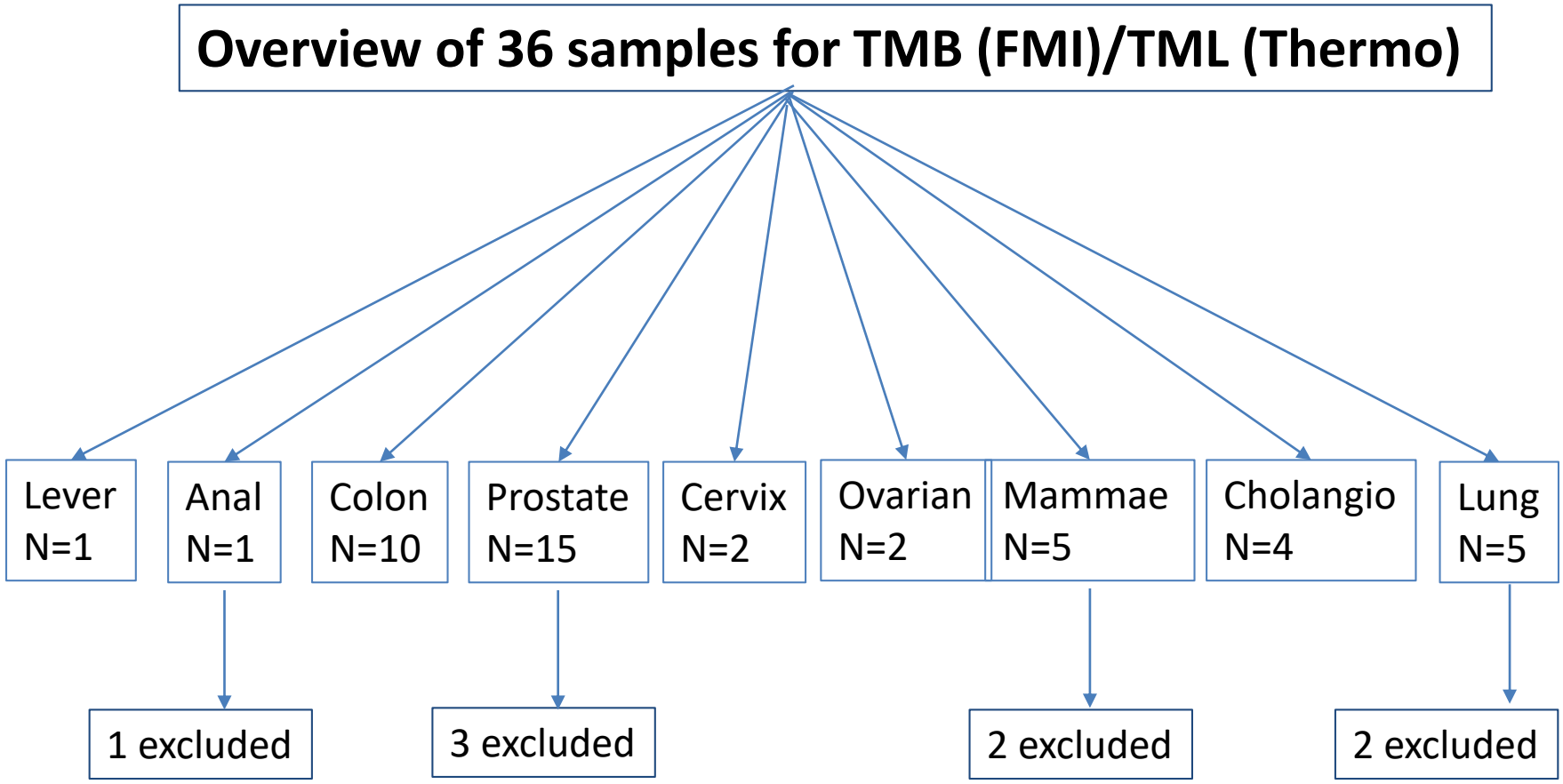


8 excluded for TMB (FMI) as there were not enough tissue

1 result with in-complete report (TMB/FMI)

36 results with complete report (TMB/FMI and TML/Thermo)

Results for all 45 samples using Thermo


Overview of 36 samples for TMB (FMI)/TML (Thermo)
Lever N=1
Anal N=1
Colon N=10
Prostate N=15
Cervix N=2
Ovarian N=2
Mammae N=5
Cholangio N=4
Lung N=5
1 excluded
3 excluded
2 excluded
2 excluded

Rapport from FMI (MSI, mutations and TMB)

FOUNDATIONONE®

Patient Name	Report Date	Tumor Type
[redacted], DK	18 January 2018	Colon adenocarcinoma (CRC)

Date of Birth	03 August 1967	**Medical Facility**	Herlev Hospital		
Sex	Female	**Ordering Physician**	[redacted]	**Specimen Received**	03 January 2018
FMI Case #	[redacted]	**Additional Recipient**	Not Given	**Specimen Site**	Abdomen
Medical Record #	Not Given	**Medical Facility ID #**	[redacted]	**Date of Collection**	05 April 2017
Specimen ID	FMI 29	**Pathologist**	Estrid Hoegdall	**Specimen Type**	Block

ABOUT THE TEST:

FoundationOne™ is a next-generation sequencing (NGS) based assay that identifies genomic alterations within hundreds of cancer-related genes.

PATIENT RESULTS

26 genomic findings

13 therapies associated with potential clinical benefit

0 therapies associated with lack of response

51 clinical trials

TUMOR TYPE: COLON ADENOCARCINOMA (CRC)

Genomic Alterations Identified†

ERBB2 V842I
ERBB3 V104M
PTCH1 N97fs*20
CDK12 G1271fs*23
PTEN D268fs*30, S10N
RNF43 G659fs*41
SMARCB1 R377C
APC D1636fs*2
ASXL1 G645fs*58
BCOR K839fs*17

Mutation in FMI and Thermo

- Genepanels in the two assay are not identical
- Concordant genes same results – if looking at raw-data

Expected to give some differences in number

TMB/TML….

- Different algorithms
- Different genes
- Different definitions of what should be filtered out

OM 40

Why in panel? Alternative transcript?

From FMI report

From TML

	FMI	.	HERLEV	NOTE
GEN	MUTATION	GEN	MUTATION	
MPL	p.Arg102Pro	MPL	p.Arg102Pro	
DICER	p.Ala20Val	DICER	p.Ala20Val	
PRDM1	p.Ala52Asp	PRDM1	p.Ala52Asp	
AR	p.His875Tyr	AR	p.His875Tyr	
FLT1	p.Ser733del			Not covered
CEBPA	p.Pro196_Pro197insHis			NOT IN PANEL
RPTOR	p.Arg369Gly			NOT IN PANEL
RPTOR	p.Arg849His			NOT IN PANEL
SPOP	p.Asp130Asn			NOT IN PANEL
NOT IN PANEL		TRRAP	p.Ala1095Thr	
		FLT3	p.Ala813Ala (=)	
NOT IN PANEL		LTF	p.Arg291Cys	
NOT IN PANEL		PDGFB	p.Arg224Trp	
NOT IN PANEL		ERCC5	p.Gly926Gly (=)	
7 Muts/MB			5.4 Muts/MB	

Cut-off, the right one?

FMI: Mutation frequence =10%? Herlev 5%

OM 256

	FMI	HERLEV	3	NOTE
GEN	MUTATION	GEN	MUTATION	
CD79A	p.THR140Asn			Missing
CDK12	p.Arg1333His	CDK12	p.Arg1333His	
PIK3C2B	p.Phe1420Leu	PIK3C2B	p.Phe1420Leu	
PTPN11	p.Pro107Arg	PTPN11	p.Pro107Arg	
SMO	p.Arg290Cys	SMO	p.Arg290Cys	
CDK12	p.His1035fs*22	CDK12	p.His1035fs	
ARID1B	p.Val1256Leu			NOT IN PANEL
CDK12	p.Leu811Arg			not covered
CYLD	p.Asn300Ser			not covered
FAT1	p.Arg628Gln			NOT IN PANEL
		ARID1A	p.Pro1898fs	
		MUTYH	p.Ala64fs	
NOT IN PANEL		PDE4DIP	P.Asp1416Asn	
NOT IN PANEL		PDE4DIP	p.Glu760fs	
NOT IN PANEL		MARK1	p.Arg623His	
NOT IN PANEL		ITGA9	p.Ala325fs	
		FGFR3	p.Pro573fs	
NOT IN PANEL		EPHB4	p.Gln851Ter	
NOT IN PANEL		CSMD3	p.Tyr1575His	
NOT IN PANEL		TAF1L	p.Arg1138Cys	
NOT IN PANEL		NOTCH4	p.Thr496Thr (=)	
		IGF2	p.Leu136Leu (=)	
NOT IN PANEL		COL1A1	p.Gly383Gly (=)	
		TAF1	p.Glu1319Glu (=)	
NOT IN PANEL		PIK3C2B	p.Pro1461Pro (=)	
6 Muts/MB			12.1 Muts/MB	

Not the same genes in the assay, some genes are not covered but are in raw-data

But how to use the number correct in treatment decision?

Conclusion

- The results are not identical and need to be examined before use in clinical setting
- Called SNP (and indels) should be re-analysed
- Studies ensuring result to reflect clinical end-point is needed

To be considered and reflected:

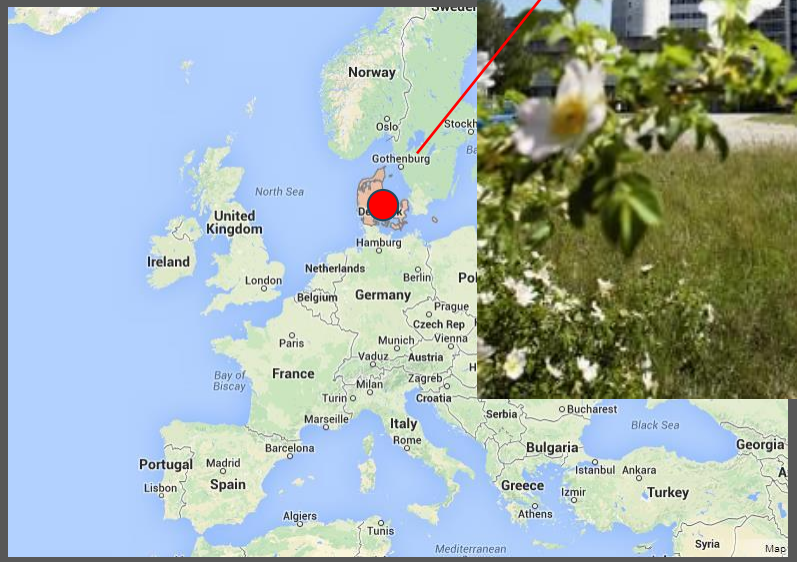
- A close colaboration beween laboratory and company is needed to optimize TML/TMB before results may truely predict response of immunotherapy

Herlev and Gentofte Hospital
Department of Oncology

May help us to understand the right biomarker algorithm for TMB/TML and thereby the right cut-off

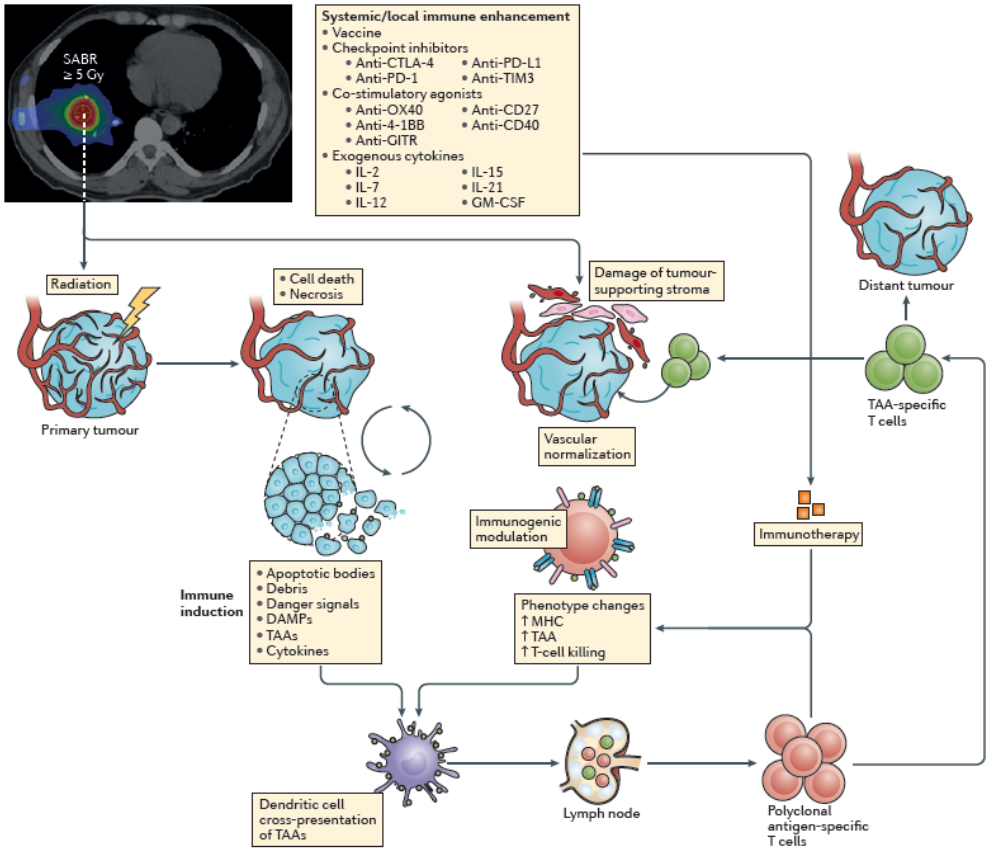
REGION

'CheckPRO trial: Randomised phase 2 trial with immunotherapy with or without stereotactic radiation therapy of a soft tissue metastasis in metastatic castration resistant prostate cancer



PI:
Rikke Løvendahl Eefsen
Clinical Oncologist, PhD

The **abscopal effect** refers to the ability of localized radiation to trigger systemic antitumor effects.



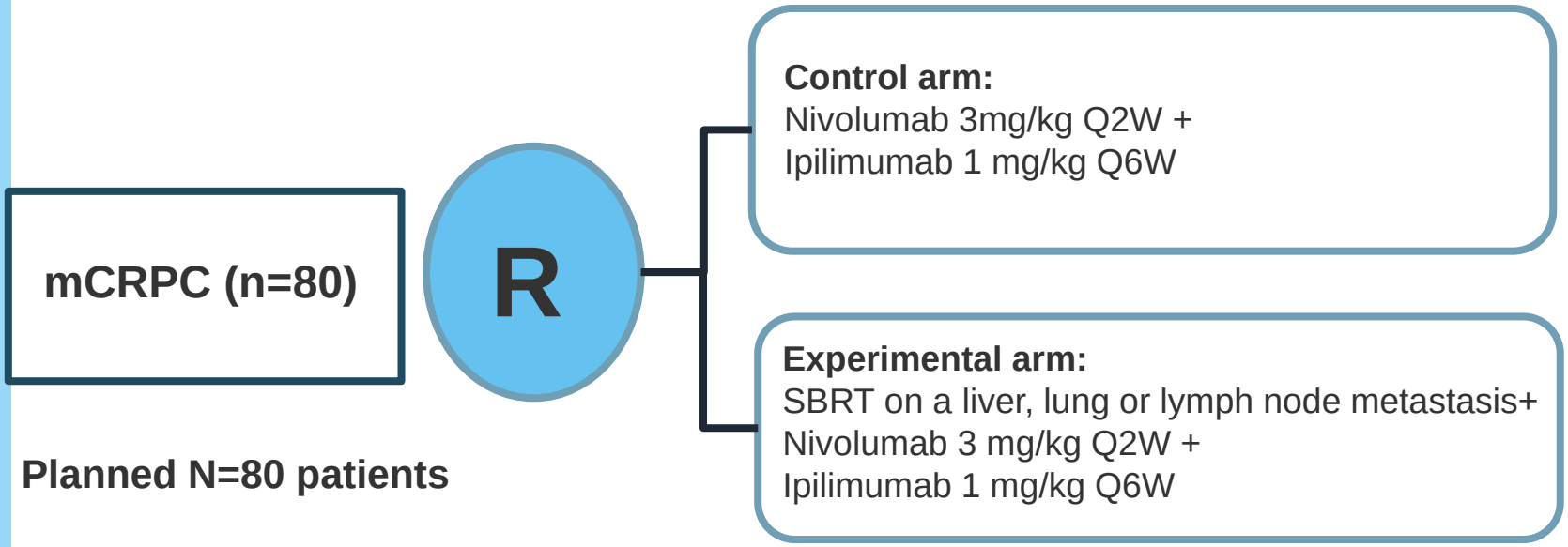
Bernstein MB et al, Nature 2016

Study objective

- The objective of this trial is to investigate **efficacy, safety and biomarkers** in patients with **prostate cancer**, who receive radiation therapy on soft tissue metastasis in liver, lung or lymph node in combination with check point inhibitors (nivolumab 3mg/kg Q2W + ipilimumab 1 mg/kg Q6W) versus check point inhibitors alone.

- Therapy in the experimental arm is proposed to generate an ”ascopal” effect, where radiation therapy induces an inflammatory response, which is expected to be more pronounced by the combination with check point inhibitors.

Study Design



Planned N=80 patients

Primary endpoint: CBR (CR, PR, SD) by RECIST 1.1.
Secondary endpoints: PFS, ORR by RECIST 1.1, DoR, irRECIST, OS at 6 months and 1 year, safety, exploratory biomarker analyses

REGION H

Stratification: TMB

Exploratory biomarker analyses

TRANSLATIONAL:

Tissue:

Biopsy taken before and after 3 months therapy:

NGS: TML (Thermo), mutations,

IHC: CD8+, CD4+, CD3+, PD-L1, LAG-3,

Treg, MDSC, TAM, ADAMs, TILs

Blood (plasma, whole blood and serum):

TML (Thermo), ctDNA, cytokines, interleukines, metabolites

Timeline

- Estimated enrollment time 24 months.

By this study we will learn:

1. **the right TMB cut-off for patients diagnosed with prostate cancer**
2. **if TMB cut-off is equal or different for tissue and blood**
3. **the true TMB cut-off for prostate cancer based on clinical end-point**

Collaborators

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MD, PhD Poul Geertsen

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BRIC: Biotech Research &Innovation Centre
Copenhagen University
Denmark

REGION H

CENTER FOR CANCER IMMUNE THERAPY

BRIC Biotech Research & Innovation Centre

Phase I unit – now, combined analyses

M-PARP (PARP_kfe) – genome instability/mutations:
Oncomine comprehensive (DNA +RNA)
BRCA1/2
HRR
MSI – fragment analyses

M-IMMUN (IMMUN kfe):
Oncomine comprehensive (DNA +RNA)
TML (TMB)
MSI – fragment analyses

M-KOMBI (KOMBI kfe):
Oncomine comprehensive (DNA +RNA)
BRCA1/2
HRR
TML (TMB)
MSI – fragment analyses

To consider: U133 Array (PAM50), WGA, Exome, Cancer hot spot

REGION 4

Third generation sequencing – Nanopore/BIOPAC… on going

Opsummering af resultater

Cancer-associerede varianter:
SETD2 (c.6169C>T, p.Pro2057Ser)
NOTCH1 (c.4031C>T, p.Thr1344Met)
SLX4 (c.4597G>A, p.Ala1533Thr)

Chromosome ændring:
Ingen identificeret

Gen-fusioner:
Klinisk relevant fusion ikke identificeret

Tumor procent: 40%	MMR expression: ND
Mutationsload: 8,45	Mikrosatelit-stabilitet: MSS
Gen status BRCA1/2: Ingen klinisk relevante	Gen status HRRm: RAD54L (c.1598G>A, p.Cys533Tyr) i exon 15
HRD status: ND	Relevant IHC: ND

Variants of unknown significance (VUS)

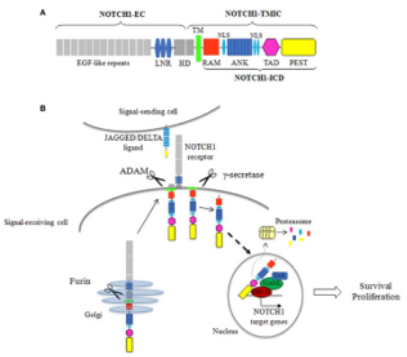
PIK3C2B	*c.745T>G*	*p.Leu249Val*	*missense*
MARK1	*c.1943C>T*	*p.Thr648Met*	*missense*
FN1	*c.6047C>T*	*p.Pro2016Leu*	*missense*
PIK3CB	*c.692G>A*	*p.Arg231His*	*missense*
SYNE1	*c.19318T>C*	*p.Phe6440Leu*	*missense*
SAMD9	*c.1058C>T*	*p.Thr353Met*	*missense*
NUP98	*c.1288T>A*	*p.Ser430Thr*	*missense*

Gen variant: NOTCH1 (c.4031C>T, p.Thr1344Met) i exon 25

Allel frekvens: 37,4%

Klassifikation: Likely Benign

Class:



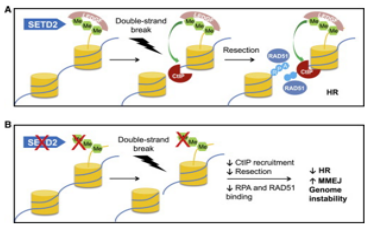
NOTCH1 is a transmembrane receptor that participates in an evolutionarily conserved cell-to-cell signal transduction pathway (PMID: 24651013). Interaction of the NOTCH1 receptor with ligand molecules on adjacent cells results in the proteolytic cleavage of NOTCH1 by gamma-secretase (PMID: 24651013). The cleaved intracellular NOTCH1 domain can then activate gene expression in the nucleus and regulate various aspects of cell differentiation, growth, proliferation, survival, and metabolism (PMID: 27507209). The specific effects of NOTCH1 signaling vary depending on the cellular context (PMID: 21508972, 24651013). NOTCH family members are frequently mutated in a variety of cancers, and these mutations can be either gain- or loss-of-function mutations (PMID: 21948802). Translocations and activating mutations in NOTCH1 have been identified in T-cell acute lymphoblastic leukemia (T-ALL), chronic lymphocytic leukemia, and adenoid cystic carcinoma (PMID: 15472075, 24170027, 27870570). These NOTCH1 activating mutations either enhance the

Gen variant: SETD2 (c.6169C>T, p.Pro2057Ser) i exon 14

Allel frekvens: 48,2%

Klassifikation: Likely benign

Class:



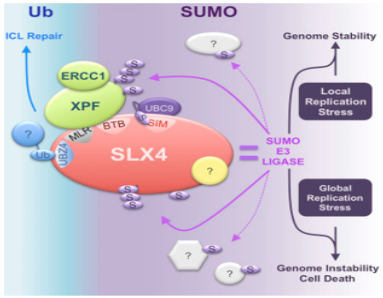
SETD2 encodes a chromatin modulating enzyme that functions by site specific trimethylation of histone H3K36. It was originally identified as a contributing enzyme in the pathogenesis of Huntington Disease and thus was initially named Huntington Interacting Protein B (HYPB) (PMID: 9700202). Histone methylation is a highly controlled biological process that regulates gene expression by altering the ability of RNA polymerase II to interact with DNA and thus initiate transcription (PMID:16118227, 25123655). Additionally, the SETD2-regulated H3K36 histone mark has been shown to play a role in regulating DNA mismatch repair. This suggests that inactivation of this protein can lead to enhanced genetic instability, enrichment of nonsense and frameshift mutations and ultimately oncogenic transformation of cells (PMID: 23622243, 25123655, 25728682, 24931610). Importantly, SETD2-mutant renal tumors failed to activate the p53 tumor suppressor, thus providing an alternative pathway for the inactivation of p53 that leads to defects in DNA damage repair (PMID: 24843002).

Gen variant: SLX4 (c.4597G>A, p.Ala1533Thr) i exon 12

Allel frekvens: 16,5%

Klassifikation: Likely Benign

Class:



The SLX4 protein is involved in various processes related to DNA damage repair. SLX4 localizes at double-strand breaks (DSB) on DNA where it forms a multi-protein complex by recruiting proteins involved in DNA repair and genome stability, such as ERCC1/ERCC4 and SLX1 endonucleases, MSH2/MSH3 mismatch repair complex, and telomeric TRF2, among others (PMID: 19596235, 19596236, 19595721, 19595722). SLX4 is essential for several types of DNA repair including DNA interstrand crosslinks (ICLs), Holliday junction (HJ) resolution and telomere homeostasis (PMID: 24938228). The SLX4 protein is mutated at low frequencies in various tumors, and germline mutations in the gene are the cause of a subtype of Fanconi anemia (FANCP) (PMID: 21240275, 21240277). SLX4 was studied as a putative genetic factor in familial non-BRCA1/2 breast cancer patients, but several studies failed to demonstrate its contribution (PMID: 22911665, 22401137, 21805310, 23211700).

Anvendte assay

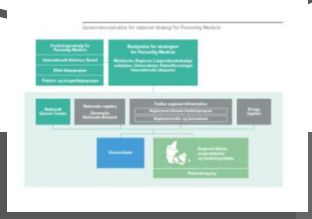
Oncomine Comprehensiv assay v.3 Oncomine BRCA1/2 Oncomine Tumor mutational Load mHRR (customer assay)	*Ion reporter 5.10*

CPR

Biobanks

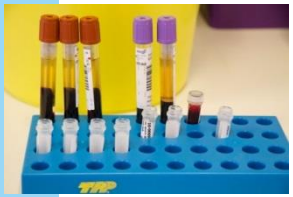
Clinical databases

Other sources



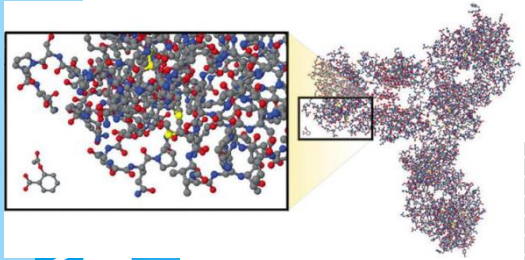
REGION

Biological materials

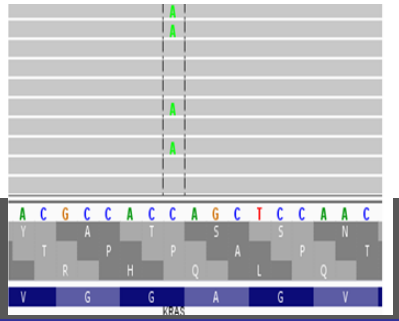



Optimal handling, Storage and data registration

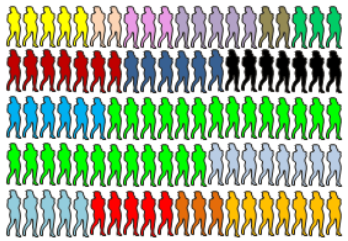
researchers



Laboratory analyses

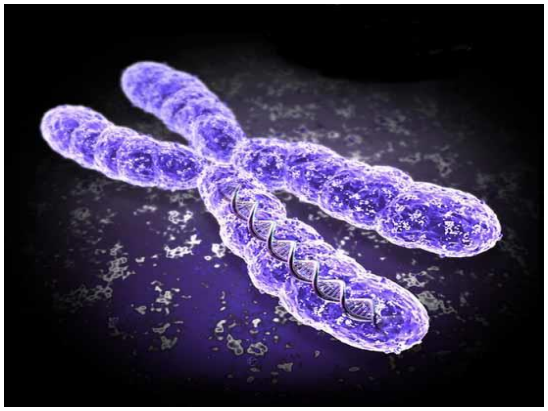


The combination of biological materials with clinical information, knowledge of new treatments may together contribute to faster implementation of personal medicine




Collaboration

Take home message:
Doing now what the patients need next



Questions?