Genome Sequencing in Cancer Research and Therapy

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Genome Sequencing in Cancer Research and Therapy

Stefan Fröhling

National Center for Tumor Diseases (NCT) Heidelberg
German Cancer Research Center (DKFZ)
Heidelberg University Hospital

IMBS Symposium: Science, Ethics and Society

Freiburg, August 28, 2015

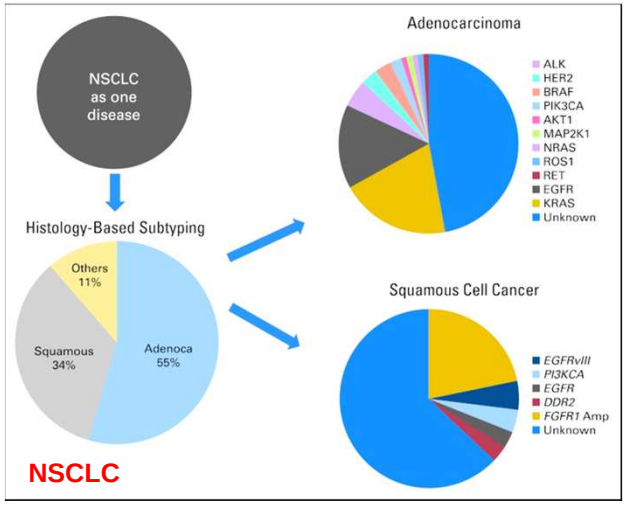
dkfz.
50 Jahre – Forschen für ein Leben ohne Krebs

UniversitätsKlinikum Heidelberg

NCT

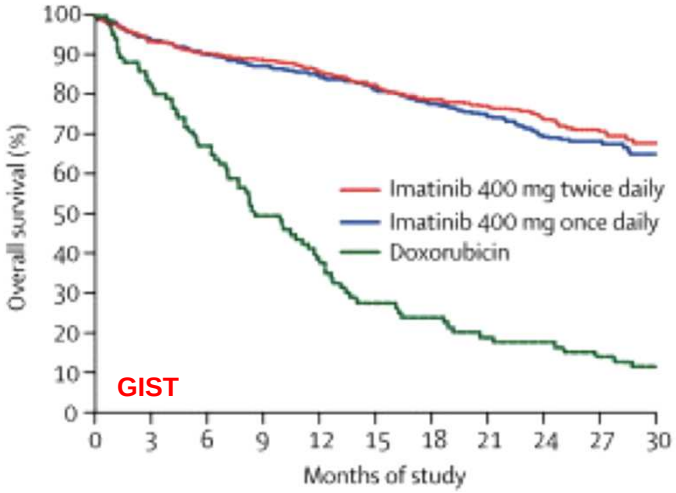
Cancer Genome Sequencing

Pathogenetic Insights and Clinical Impact



Common cancers as multiple rare diseases of the same organ, demanding unique therapies

Li et al. J Clin Oncol 2013



Improved clinical outcome through genotype-directed therapy

Verwej et al. Lancet 2004

Distinct mutations shared across multiple cancers

BRAF$^{V600E/K}$ in melanoma; thyroid, lung, colorectal, ovarian, gastric, esophageal, head and neck cancer; gastrointestinal stromal tumor; glioma; hairy-cell leukemia; multiple myeloma; etc.

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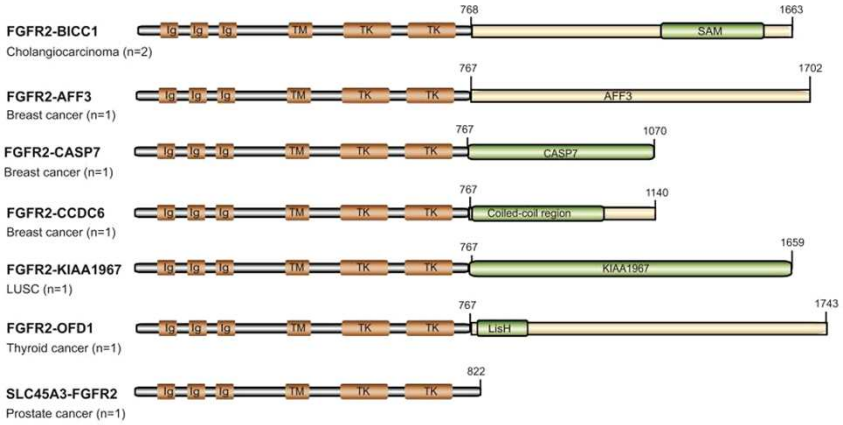
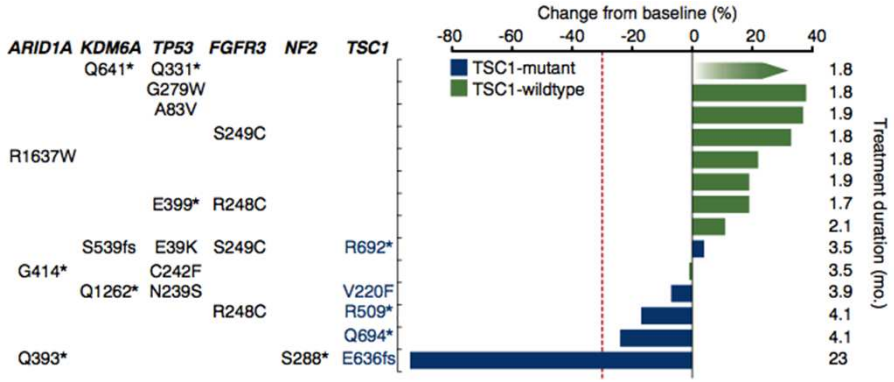
Cancer Genome Sequencing

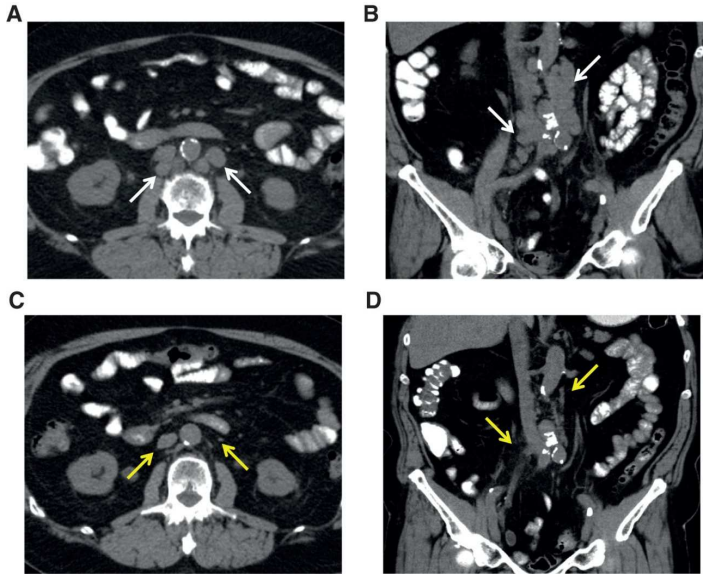
"N = 1" Studies



Targetable FGFR fusions in diverse cancers

Wu et al. Cancer Discov 2013





Activating mTOR mutations in urothelial carcinoma

Wagle et al. Cancer Discov 2014

Inactivating TSC1 mutations in urothelial carcinoma

Iyer et al. Science 2012

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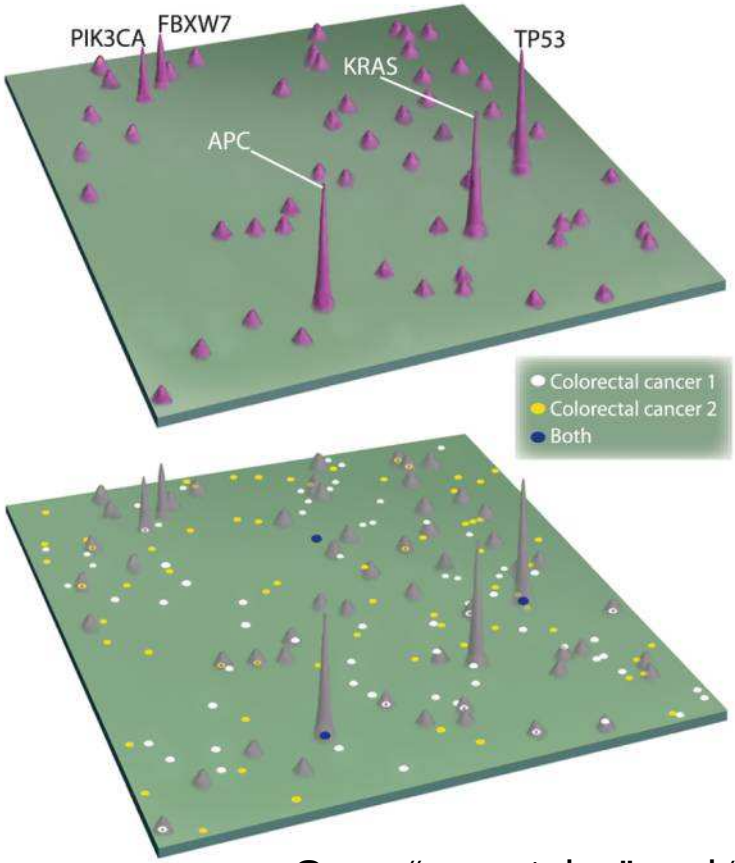
Cancer Genome Sequencing

Importance of Rare Mutations

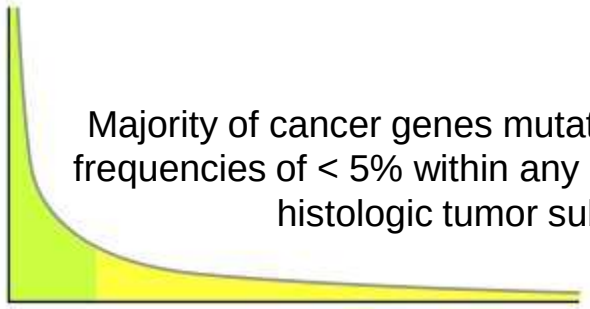


Gene “mountains” and “hills”

Wood et al. Science 2007

The Cancer Genome Atlas

Understanding genomics to improve cancer care



Majority of cancer genes mutated at frequencies of < 5% within any given histologic tumor subtype

“Long tail” pattern of actionable cancer gene alterations

TCGA Pan-Cancer Analysis
Lawrence et al. Nature 2013

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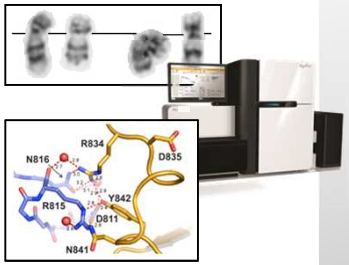
Molecularly Aided Stratification for Tumor Eradication Research

Molecular diversity and genetic taxonomy of cancer

Individual, "private" patterns of molecular lesions

Genetics



Actionability of molecular lesions

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Registry Trial

Clinical care

- Prognostic / predictive markers
- Therapeutic targets

Molecularly stratified clinical trial(s)

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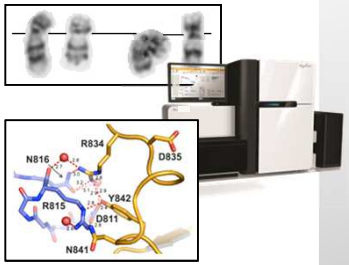
Eligibility and Objectives

Genetics



Clinical care

Eligibility

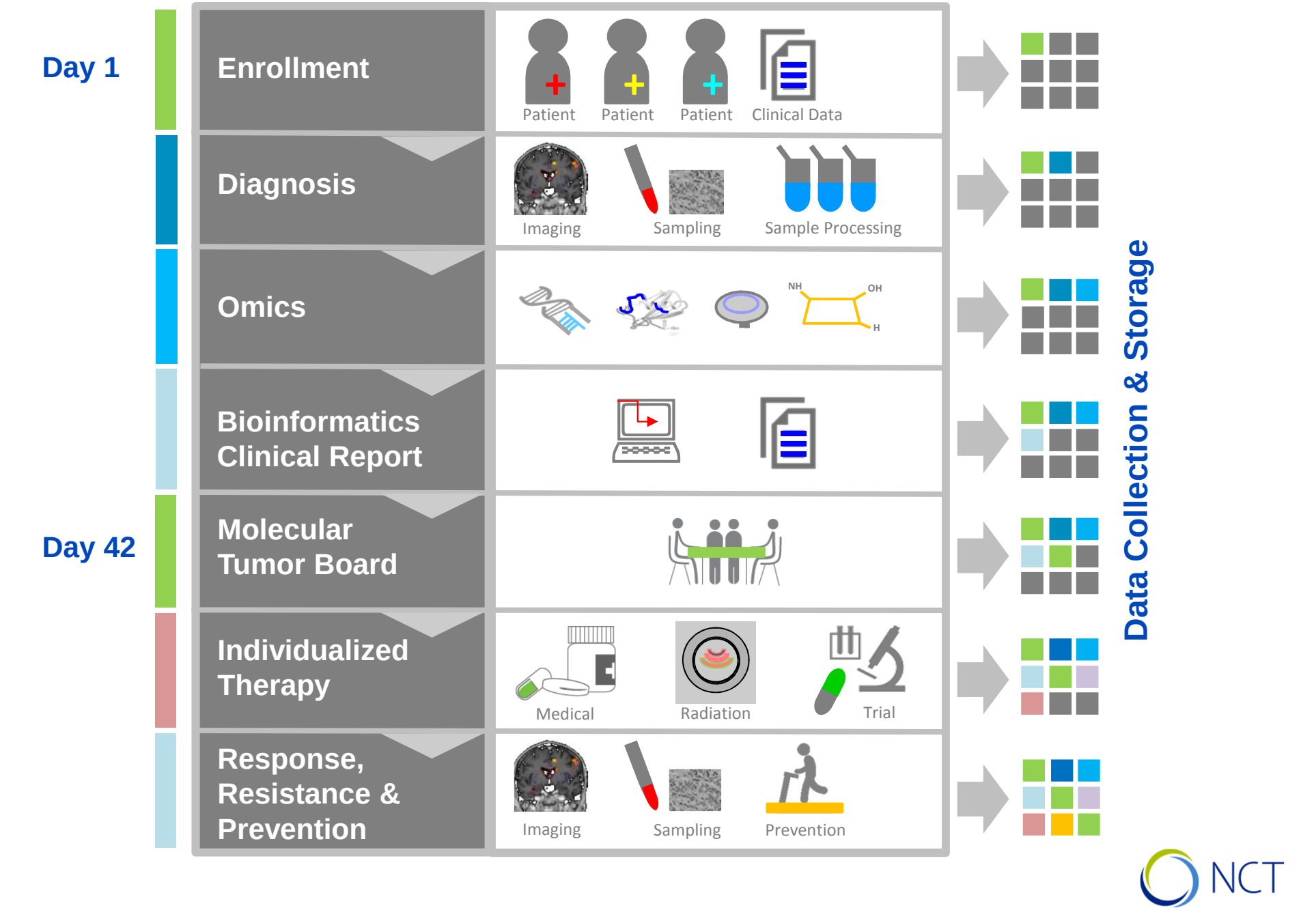
- Patients younger than 51 years
- Patients with rare cancers
  - Incidence of less than 1/100,000 per year
- Measurable disease activity
- No curative treatment available
- Karnofsky Performance Status of at least 70%
- Life expectancy of at least 6 months

Objectives

- Prospective exome and transcriptome sequencing within clinical context
- Interdisciplinary evaluation and formulation of treatment recommendations
- Translation into individualized patient care

DKFZ-HIPO

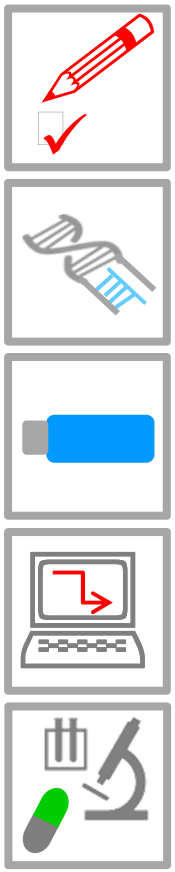
NCT POP
Precision Oncology Program


Day 1
Day 42
Enrollment
Patient
Patient
Patient
Clinical Data
Diagnosis
Imaging
Sampling
Sample Processing
Omics
NH
OH
H
Bioinformatics
Clinical Report
Molecular
Tumor Board
Individualized
Therapy
Medical
Radiation
Trial
Response,
Resistance &
Prevention
Imaging
Sampling
Prevention
Data Collection & Storage

Enrollment

NCT MASTER Protocol

Umbrella protocol for implementing precision oncology at NCT, consenting every patient for:



- Questionnaires – Health and Behavior
- Molecular Analysis
- Data Storage
- Clinical Data Analysis
- Recontact for Clinical Trials

Diagnosis

NCT POP / DKFZ-HIPO Sample Processing Lab

SOP-Guided Preparation of Analytes

Tasks

- Sample registration and barcoding
- Sample preparation
- Nucleic acid extraction
- Quality assessment and identity check
- Sample submission to core facility
- Documentation
- Methods development and optimization
- Sample management and storage

Submissions in 2014

• Exome sequencing	958
• Whole-genome sequencing	183
• Transcriptome sequencing	298
• SNP array profiling	283
• Expression profiling	46
• Methylation profiling	164
• **Total**	**1,932**

Omics, Bioinformatics, Clinical Report

High-Throughput Sequencing Unit

Tasks

- Automated library preparation
- Highly parallel sequencing using Illumina technology
- 14 HiSeq 2000, 2 HiSeq 2500 systems

Clinical Bioinformatics

Tasks

- Automated alignment
- Variant calling, selection of somatic mutations
- Gene expression analysis
- Annotation with COSMIC, Cancer Gene Census, potential drugs and pathways

Translational Oncology

Task

- Clinical interpretation of molecular data

Molecular Pathology

Task

- Validation of actionable genetic alterations

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Precision Oncology Tumor Board

Translational Oncology

Referring Physicians

Pathology

Friday, 2:30 PM
NCT, Room K4

Bioinformatics

Sample Processing Lab

Sequencing Facility

Clinical case
- Referring physicians

General characteristics (number of alterations, mutation patterns etc.)
- Bioinformatics

Presentation of potentially actionable lesions
- Translational Oncology

Discussion of potential clinical implications
- All

Precision Oncology Outpatient Clinic
- Communication of results
- Initiation/monitoring of therapy

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Tumor Board Report

NCT NATIONALES CENTRUM FÜR TUMORERKRANKUNGEN HEIDELBERG

getragen von:
Deutsches Krebsforschungszentrum
Universitätsklinikum Heidelberg
Thoraxklinik Heidelberg
Deutsche Krebshilfe

Beschluss Tumorboard

Zweitmeinung: ☐ ja ☐ nein ☐ fehlende Angabe

Abrechnung: ☐ Poli. Beh. ☐ amb. SZ ☐ Stationär ☐ Privatpatient ☐ amb. Mitb./Nachstat.

Patient: [redacted]		**Geb.-Datum:** [redacted]
Station/Ambulanz: NCT Leitstelle	Arzt: Prof. Dr. Stefan Fröhling	Konf.-Datum: 17.01.2014
Erforderliche Facharzte		

ICD	
Erstdiagnose:	Hepatisch metastasiertes retroperitoneales LMS
Chronologie	Hepatisch metastasiertes retroperitoneales LMS; Z. n. neoadjuvanter Bestrahlung des Primärtumors, multiplen Operationen und Chemotherapie mit Doxorubicin/Ifosfamid; zuletzt palliative Chemotherapie mit Trabectedin bei inoperablen Lebermetastasen, hierunter Progress der hepatischen Metastasierung und V. a. neu aufgetretene Lungenmetastasen
Fragestellung an die Konferenz:	Therapierelevante genetische Veränderungen?

Beteiligte der Konferenz:
Translationale Onkologie: Prof. Fröhling, Dr. Heining, Prof. Glimm, Fr. Dr. Richter
DKFZ: Prof. Sültmann
Medizinische Onkologie: FOA PD Dr. Dr. Ungerechts, FOA Dr. Haag, FOA Dr. Vallet
Schlegel Nicole, Vinzens Andrea

Konferenzbeschluss:
- Nachweis einer Amplifikation des *FGFR1*-Gens, validiert mittels FISH; Rationale für den Einsatz eines FGFR-Inhibitors, nach Möglichkeit im Rahmen einer klinischen Studie (rekrutierende Phase 1-Studie in Köln und Essen, ClinicalTrials.gov: NCT01004224
- Nachweis einer heterozygoten Mutation (K218E) des Tumorsuppressorgens *PTPN12*, validiert mittels Sanger-Sequenzierung; möglicherweise assoziiert mit aberranter Aktivierung von Rezeptortyrosinkinasen, insbesondere EGFR, ERBB2 und PDGFRB; mögliche Rationale für den Einsatz von Tyrosinkinaseinhibitoren (z. B. Erlotinib, Lapatinib, Imatinib)

Verantwortlicher für den Konferenzbeschluss: Prof. Fröhling
Die durchgeführte Analyse (Exom-Sequenzierung) und die daraus abgeleitete Therapieempfehlung sind „research grade". Es handelt sich nicht um eine qualitätsgesicherte, diagnostisch belastbare Untersuchung im engeren Sinne. Ebenso ist die Wirksamkeit der genannten zielgerichteten Therapie bei der vorliegenden Erkrankungsentität nicht durch klinische Studien gesichert.

Elektronisches Dokument

dkfz.

Validation Report

UniversitätsKlinikum Heidelberg

[redacted]

Pathologisches Institut
Geschäftsführender Direktor:
Prof. Dr. med. P. Schirmacher

Pathologisches Institut | Postfach 11 21 | 69093 Heidelberg

Abt. Allgemeine Pathologie und pathologische Anatomie
Ärztlicher Direktor:
Prof. Dr. med. P. Schirmacher
Stellvertretender Ärztl. Direktor:
Prof. Dr. med. W. Weichert

Ergebnis der Mutationsvalidierung im Rahmen des Personalisierten Onkologie Programms (POP)

Patienteninformation

Name: [redacted]

Geburtsdatum: [redacted]

Zu validierende Mutation

Chromosom	Position (hg19)	Gensymbol
7		PTPN12

Typ	Referenz-Allel	Varianten-Allel	Variante	Varianten-Frequenz
SNP	A	G	p.K218E	35%

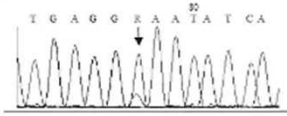
Ergebnis der Sanger-Sequenzierung:

☒ Die Mutation wurde bestätigt

☐ Die Mutation wurde nicht bestätigt

Bemerkung:

p.K218E (c.652A>G; ENST00000248594)

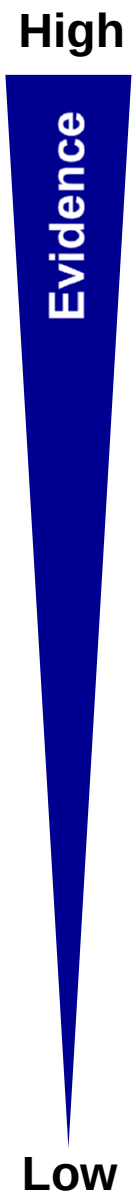


HD, 17.1.14

Ansprechpartner (Pathologie):
V. Endris (56-35596); R. Penzel (56-39907)

Die durchgeführte Analyse ist „research grade", es handelt sich nicht um eine qualitätsgesicherte, diagnostisch belastbare Untersuchung im engeren Sinne.

NCT



Level 1

A: Drug is approved for the same tumor type harboring the specific biomarker.

B: Predictive value of the biomarker or clinical effectiveness of the corresponding drug in a molecularly stratified cohort was demonstrated in an adequately powered prospective study or a meta-analysis.

Level 2

A: Predictive value of the biomarker or clinical effectiveness of the drug in a molecularly stratified cohort was demonstrated in a prospective trial with biomarkers as a secondary objective or an adequately powered retrospective cohort or case-control study in the same tumor type.

B: Predictive value of the biomarker or clinical effectiveness of the drug in a molecularly stratified cohort was demonstrated by clinical data in a different tumor type.

C: Case study or single unusual responder indicates the biomarker is associated with response to the drug, supported by scientific rationale.

Level 3

Preclinical data (in vitro or in vivo models and functional genomics) demonstrate that the biomarker predicts response of cells to drug treatment.

Level 4

Biological rationale exists that links the drug to the altered signaling pathway or relevant basket. No reported clinical or preclinical data on the response to the drug.

Adapted from:
MD Anderson Cancer Center Institute for Personalized Cancer Therapy
https://pct.mdanderson.org

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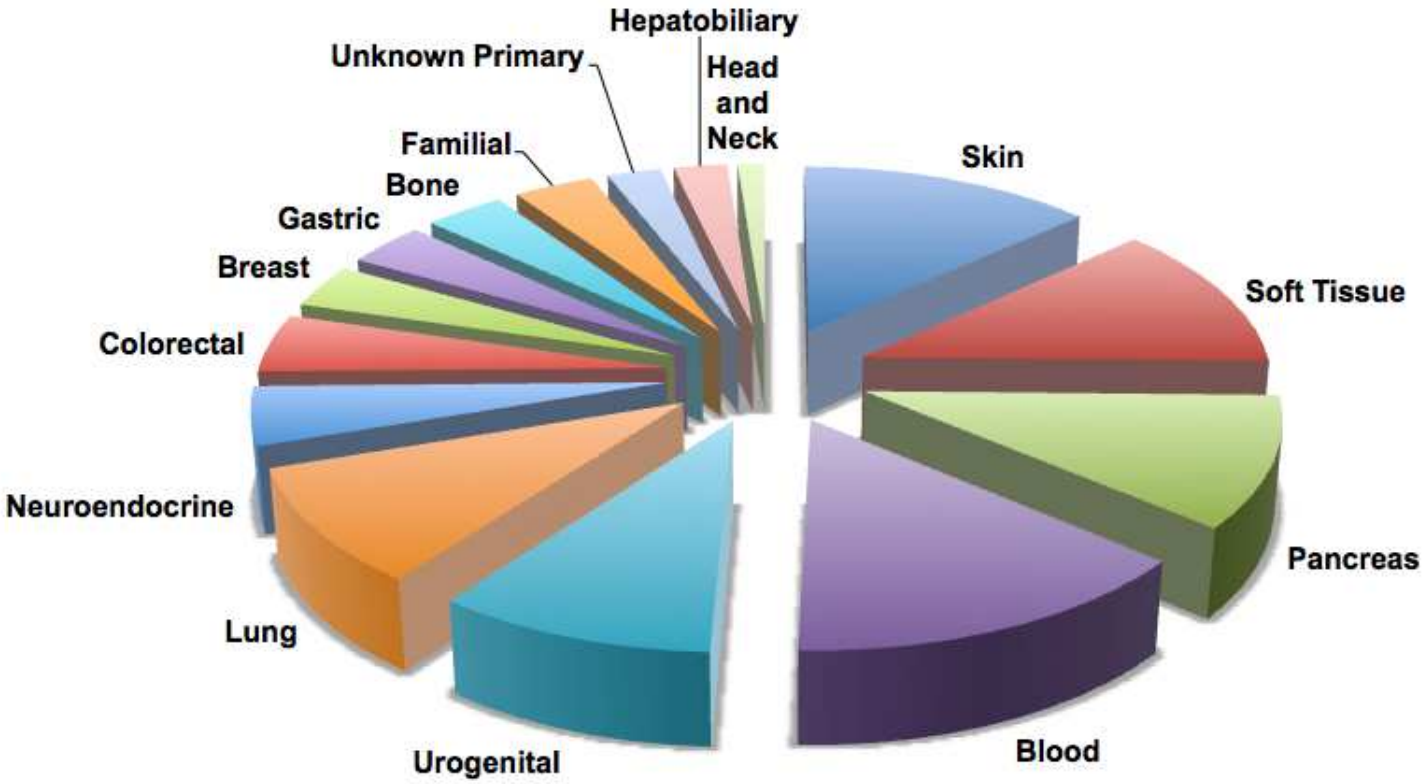
Current Status



July 2015

Registered	*N* = 384
Eligible	*N* = 322
Completed	*N* = 260
Discussed	*N* = 212
Recommendation	*N* = 125 (59%)
Treatment	*N* = 38 (18%)

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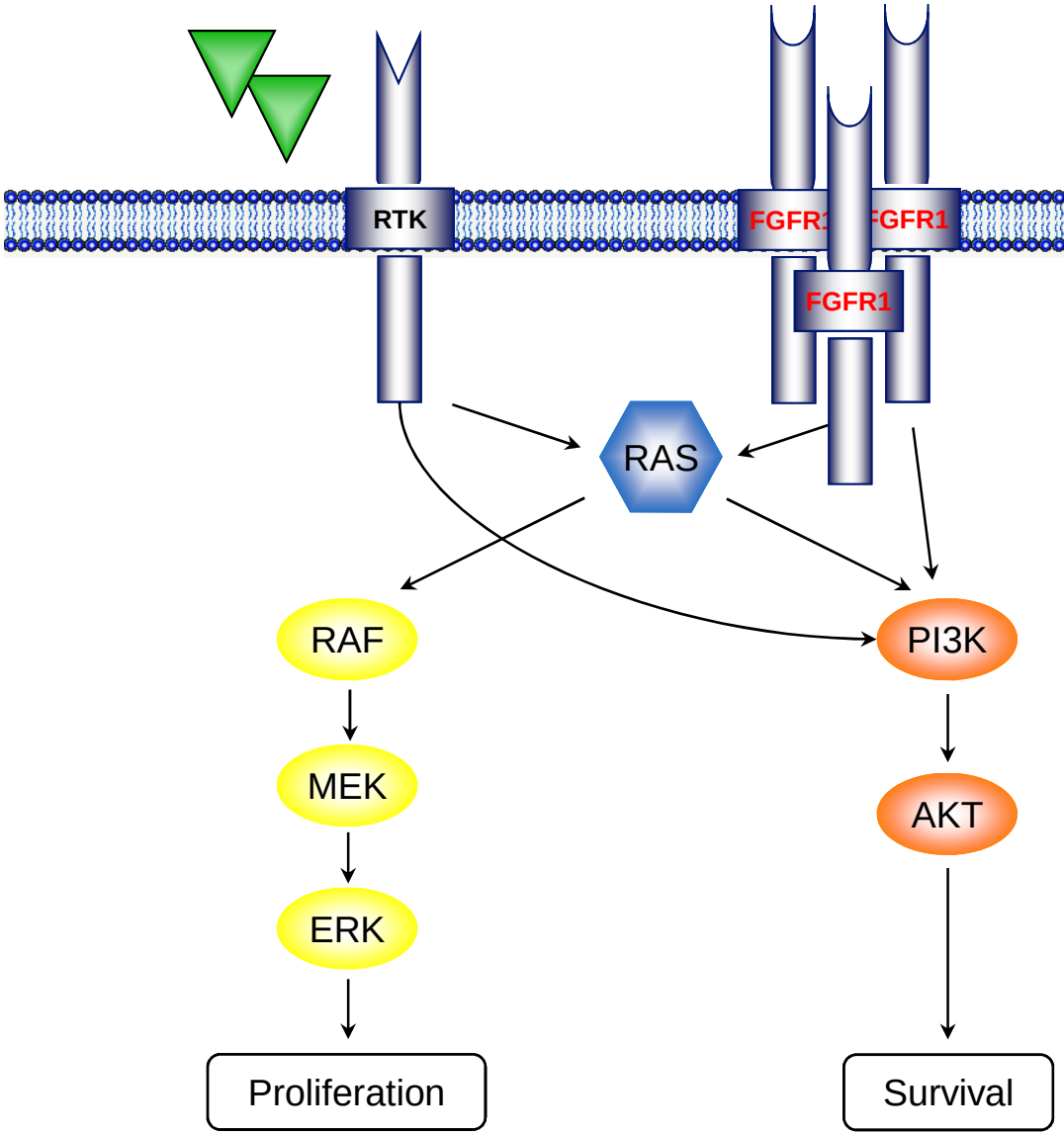
Findings With Established Clinical Implications by Histology

Disease	Mutation	Clinical Action
Melanoma	BRAF p.V600R ERBB4 p.P172F	Vemurafenib, dabrafenib Lapatinib
Uveal melanoma	GNAQ p.Q209L/P/R	Trametinib, selumetinib
Basal-cell carcinoma	PTCH1 p.648_650del	Vismodegib
Multiple myeloma	BRAF p.V600E	Vemurafenib, dabrafenib
Myxoid liposarcoma	PIK3CA p.C420R/p.E545K PTEN p.R130G	PI3K/AKT inhibitors, everolimus, temsirolimus
Gastrointestinal stromal tumor	KIT p.V560D PDGFRA p.D842V	Imatinib Crenolanib
Ovarian cancer	TSC2 p.R505X	Everolimus, temsirolimus
Breast cancer	PIK3CA p.E545K TSC1/2^{del} FGFR1^{amp}	PI3K/AKT inhibitors, everolimus, temsirolimus FGFR inhibitors
Pulmonary adenocarcinoma	EGFR p.T790M TSC2^{mut}	AZD9291 Everolimus, temsirolimus
Carcinoma of unknown primary	EGFR p.745_750del PIK3CA p.E545K	Erlotinib PI3K/AKT inhibitors, everolimus, temsirolimus

Findings With Established Clinical Implications by Gene/Pathway

Mutation	Disease	Clinical Action
BRAF p.V600R/p.V600E	Multiple myeloma Melanoma	Vemurafenib, dabrafenib
PIK3CA p.C420R/p.E545K	Breast cancer Myxoid liposarcoma Carcinoma of unknown primary	PI3K/AKT inhibitors, everolimus, temsirolimus
$TSC1^{mut}/TSC1^{del}$ $TSC2^{mut}/TSC2^{del}$	Esophageal adenocarcinoma Gastric cancer Breast cancer Ovarian cancer Pulmonary adenocarcinoma	Everolimus, temsirolimus
$FGFR1^{amp}$	Breast cancer Leiomyosarcoma T-cell prolymphocytic leukemia	FGFR inhibitors
KIT p.V560D/p.D579del	Gastrointestinal stromal tumor Sinonasal undifferentiated carcinoma	Imatinib
$ERBB2^{amp}$/p.D769Y	Colorectal cancer Cholangiocarcinoma Gallbladder carcinoma Plexiform schwannoma	Trastuzumab, pertuzumab, lapatinib, neratinib



Rationale for Experimental Targeted Treatment (1)

25-year-old patient with metastatic retroperitoneal **leiomyosarcoma**
- No local therapy options
- Refractory to doxorubicin/ifosfamide
- Refractory to trabectedin

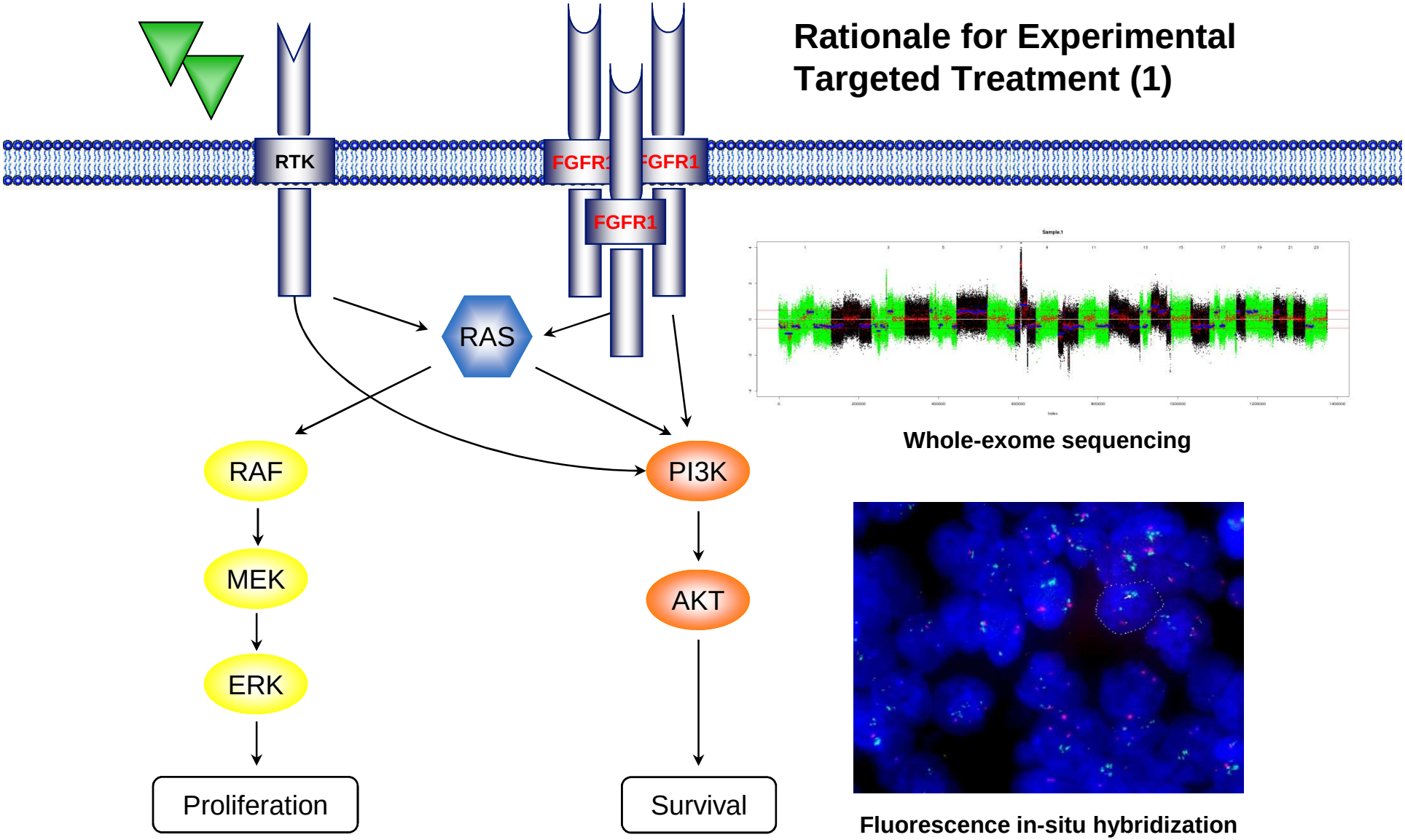
Focal **8p amplification** involving ***FGFR1***, validated by FISH

Treatment with **FGFR inhibitor** within phase 1 clinical trial for 2 months
- Discontinuation due to adverse events

Ongoing **stable disease** for >12 months with minimal metabolic activity of metastatic lesions

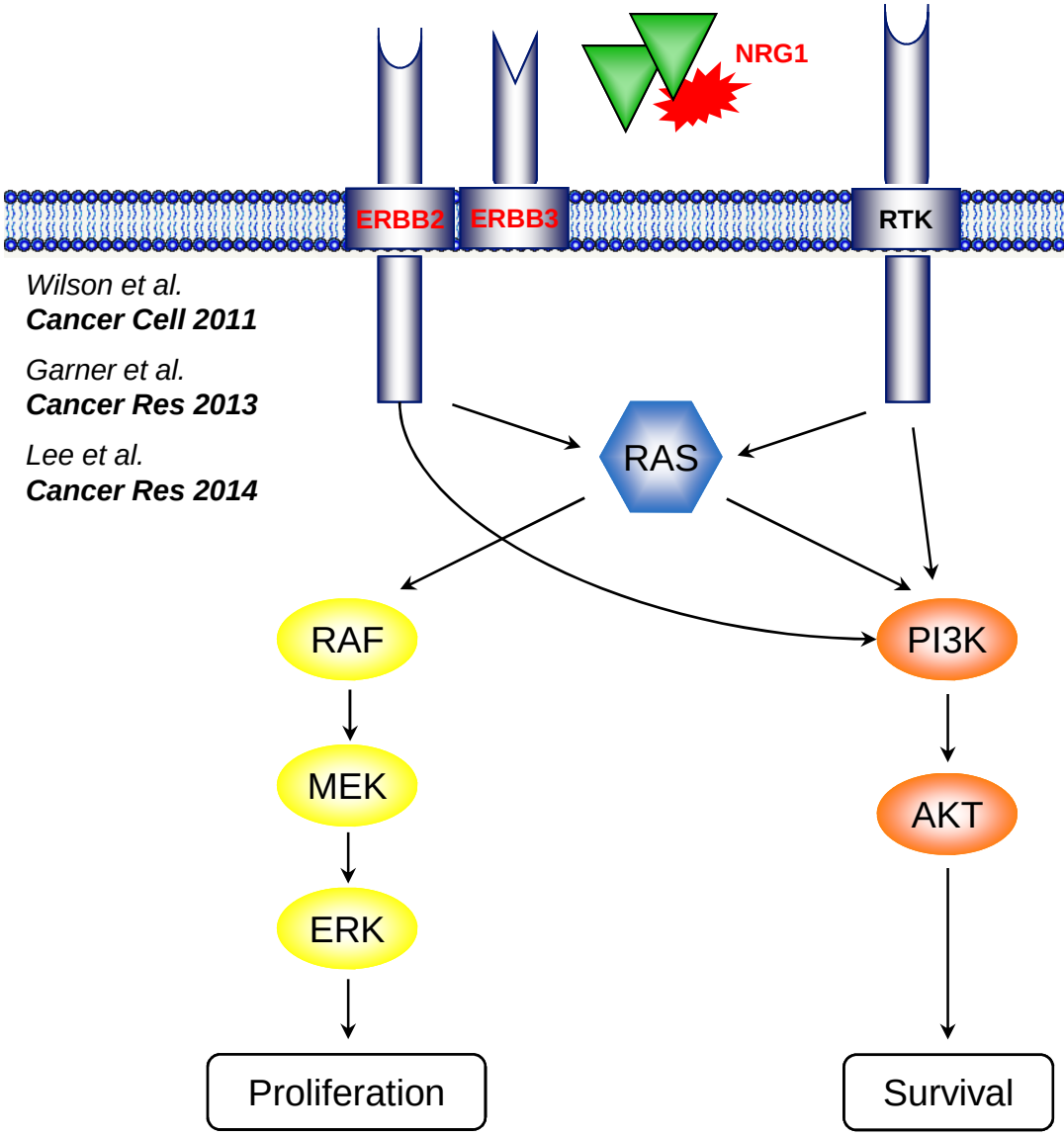
NCT MOLECULAR DIAGNOSTICS PROGRAM
Personalized Oncology at NCT – NCT MASTER

Rationale for Experimental Targeted Treatment (1)



Whole-exome sequencing

Fluorescence in-situ hybridization



Wilson et al.
Cancer Cell 2011

Garner et al.
Cancer Res 2013

Lee et al.
Cancer Res 2014

Rationale for Experimental Targeted Treatment (2)

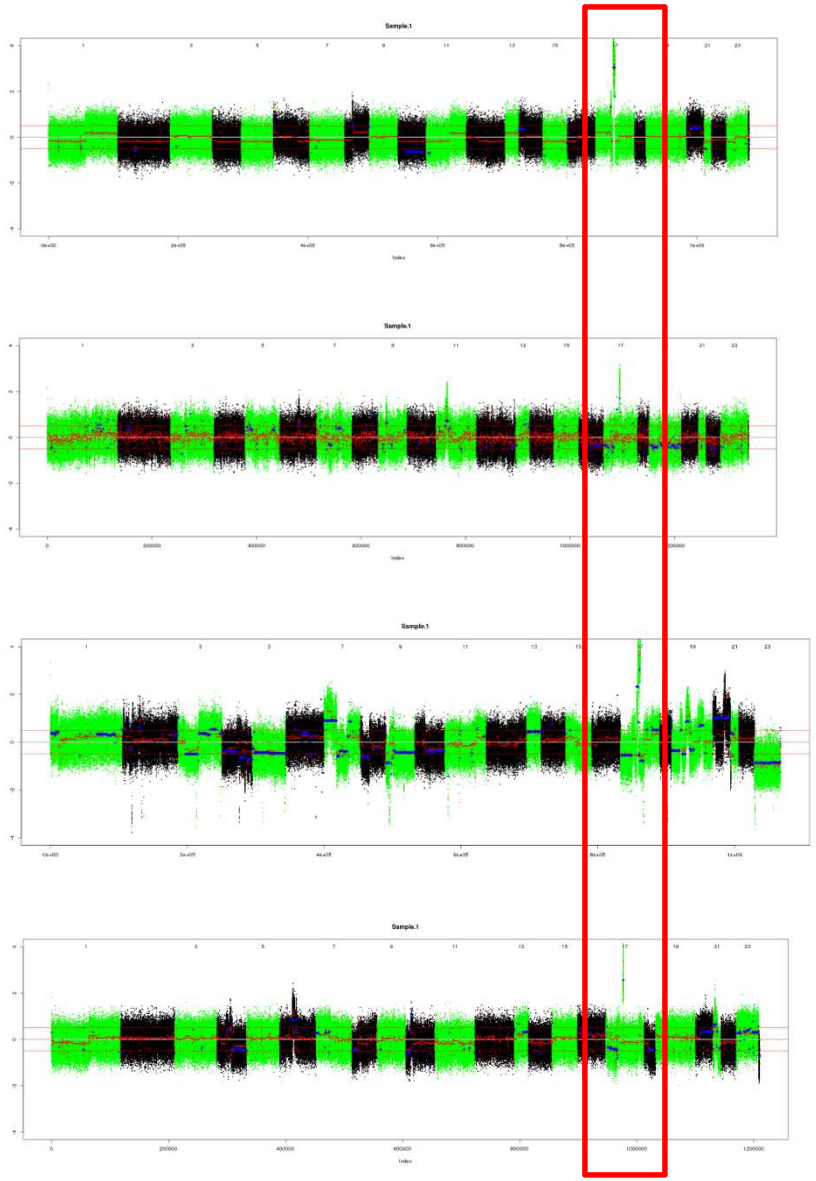
46-year-old patient with metastatic **triple-negative breast cancer**
• Refractory to multiple lines of chemotherapy

NRG1 c.1053-1G>A (splice site mutation)
NRG1 expression by immunohistochemistry

Aberrant NRG1 expression and ERBB3 activation, enhanced dimerization with other ERBB family members, and constitutive signal transduction as rationale for **pan-ERBB blockade** in “ERBB2-low” breast cancer

Treatment with **pertuzumab**, eribulin, and bevacizumab

Stable disease for >6 months



CRC

GBC*

CCC

CCC

Rationale for Experimental Targeted Treatment (3)

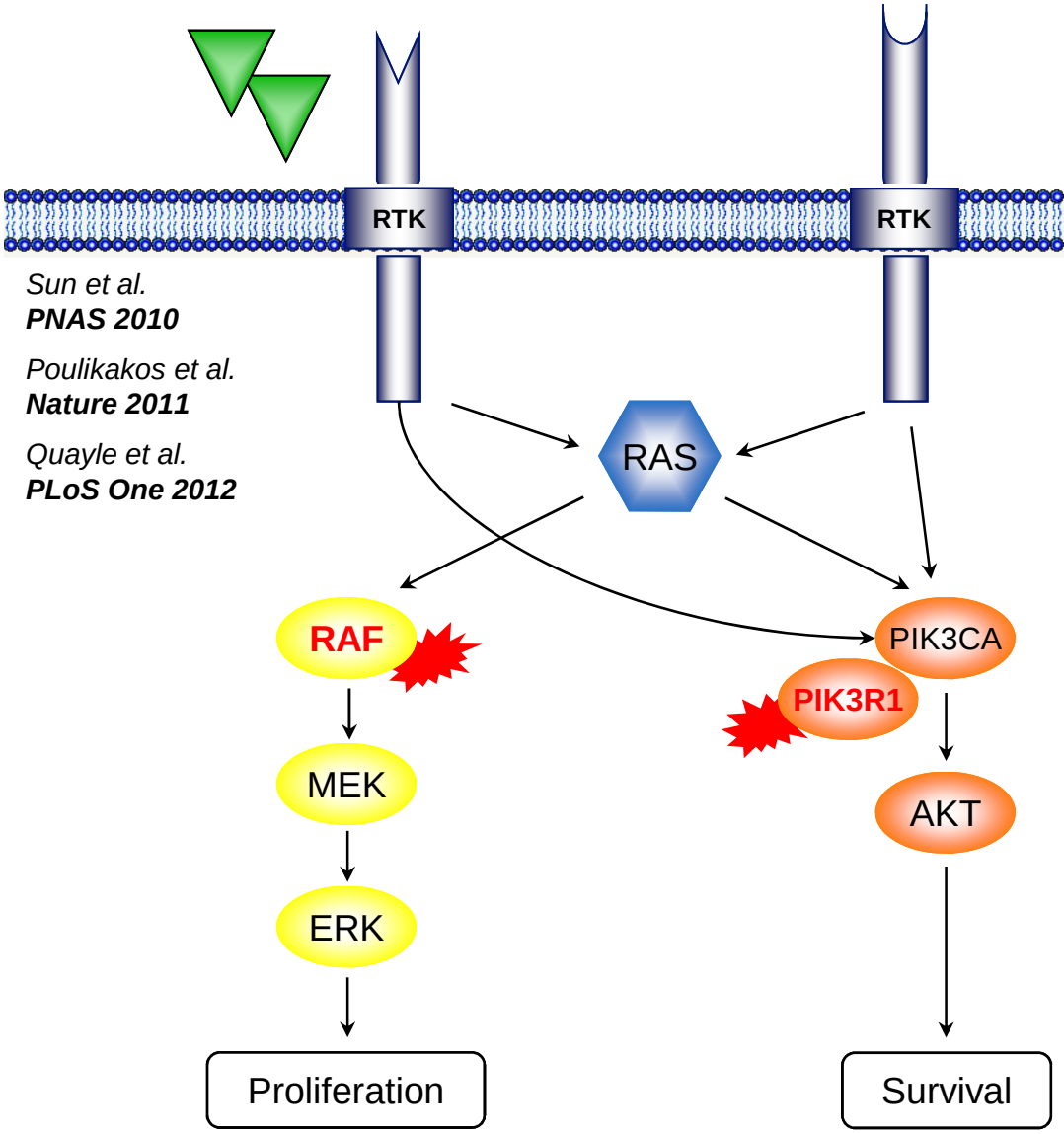
ERBB2 amplification and **overexpression** in gastrointestinal cancers
- KRAS^{WT} colorectal cancer (n=3)
- Gallbladder carcinoma (n=1)
- Cholangiocarcinoma (n=1)

Aberrant ERBB2 expression and constitutive signal transduction as rationale for **ERBB2 blockade** with trastuzumab, pertuzumab, or lapatinib

***Ongoing **partial remission** in a 37-year-old patient with metastatic **gallbladder carcinoma** treated with **trastuzumab**, **pertuzumab**, and nab-paclitaxel since 11/2014

HERACLES Trial
Trastuzumab/lapatinib in patients with heavily pretreated, ERBB2^{amp}, KRAS^{WT} colorectal cancer; DCR: 78%; median TTP: 5.5 months
Siena et al. ASCO Annual Meeting 2015

NCT MOLECULAR DIAGNOSTICS PROGRAM
Personalized Oncology at NCT – NCT MASTER



Sun et al.
PNAS 2010

Poulikakos et al.
Nature 2011

Quayle et al.
PLoS One 2012

Rationale for Experimental Targeted Treatment (6)

66-year-old patient with **glioblastoma**
- Status post incomplete resection
- Adjuvant chemotherapy with temozolomide

PIK3R1 p.G376R
- Activation of PI3K, low transforming potential

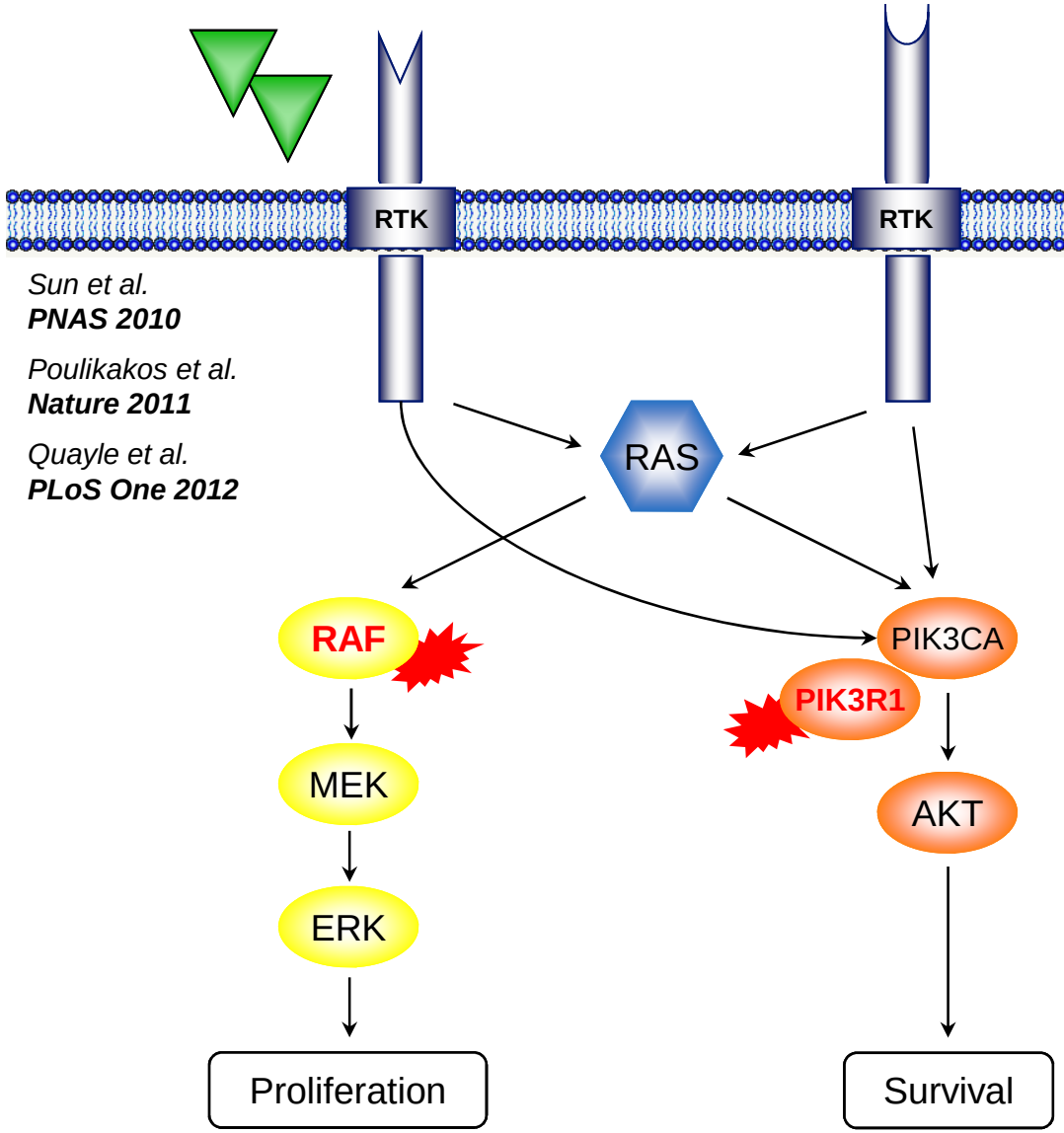
TTYH3-BRAF fusion
- Novel, structurally distinct fusion involving **near-full-length BRAF**
- BRAF activation and transforming potential

BRAF p.47_438del
- Loss of N-terminal regulatory domain and constitutive BRAF activation
- Described as **splicing alteration** associated with constitutive BRAF dimerization and **resistance to vemurafenib** in melanoma

Rationale for MAPK pathway blockade using **sorafenib** or **MEK inhibitor**

Tilman Brummer
University of Freiburg

Rationale for Experimental Targeted Treatment (6)



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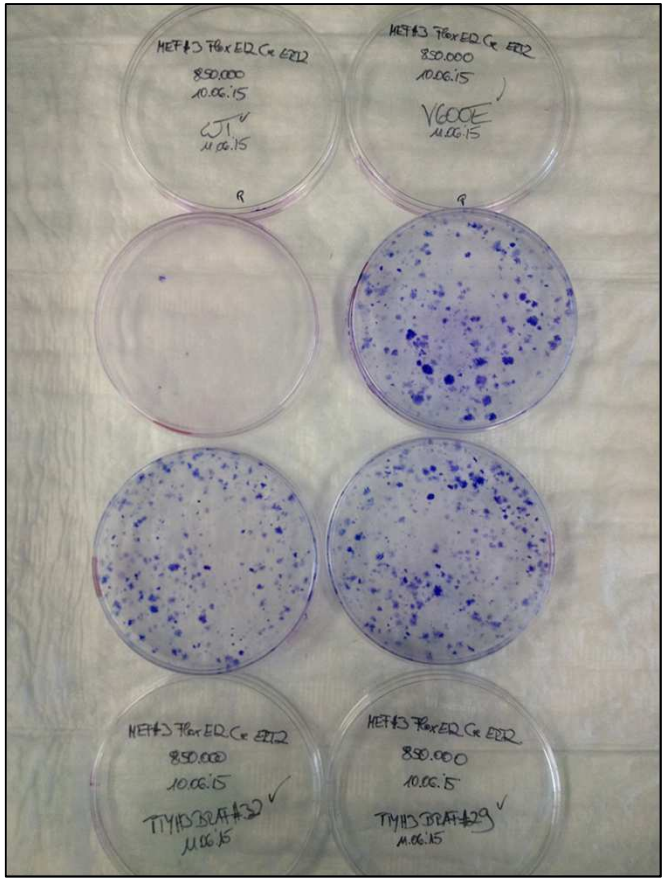

Tilman Brummer
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Rationale for Experimental Targeted Treatment (6)

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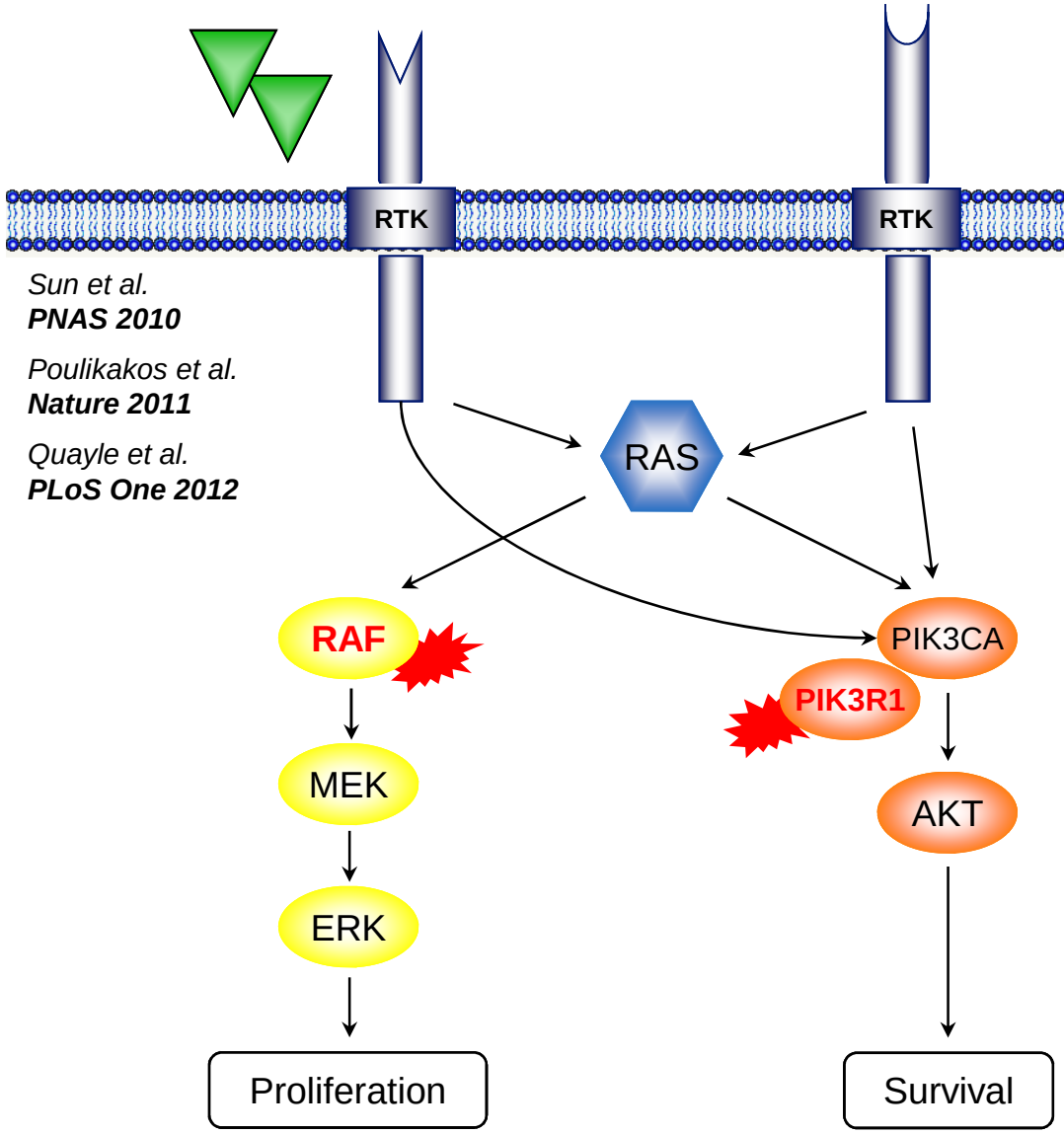
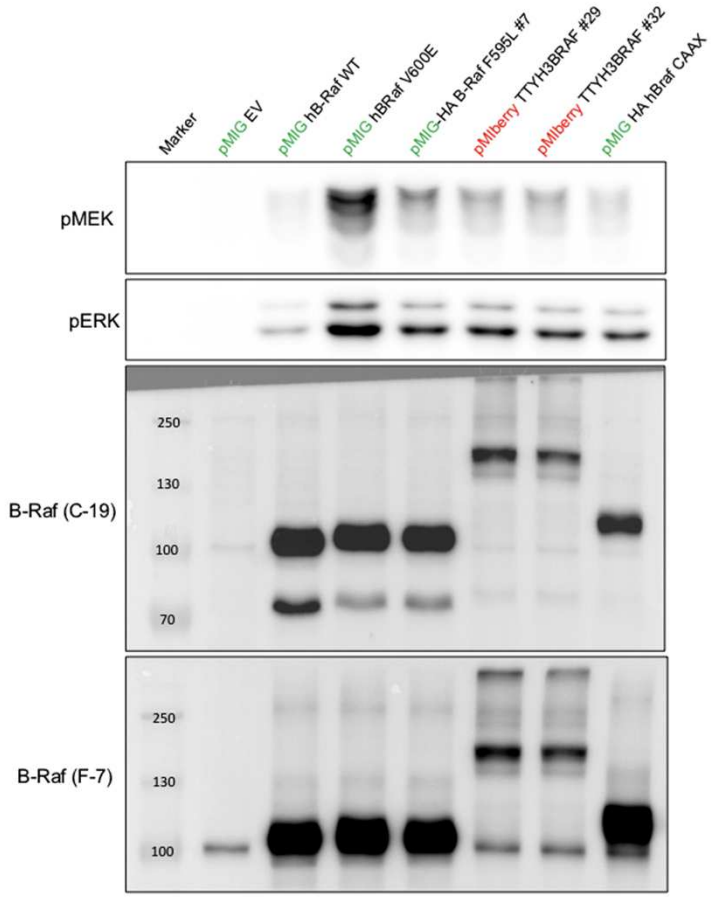
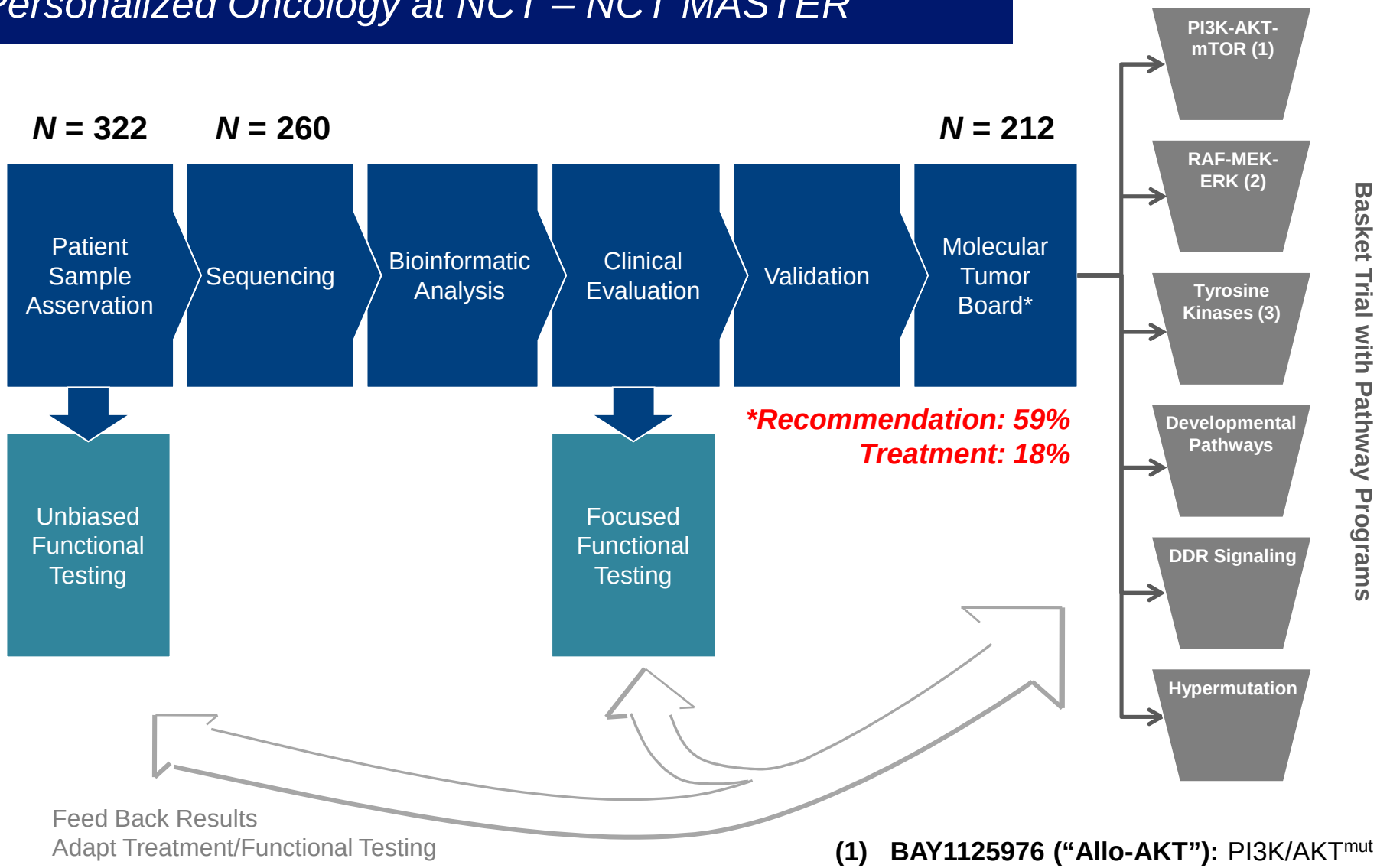




NCT MOLECULAR DIAGNOSTICS PROGRAM
Personalized Oncology at NCT – NCT MASTER



(1) BAY1125976 ("Allo-AKT"): PI3K/AKT^{mut}
(2) BRF117019 ("ROAR"): BRAF^{V600E}
(3) EORTC 90101 ("CREATE"): ALK/MET^{abn}

Stefan Fröhling, Stefan Gröschel, Christoph Heining, Hanno Glimm
Claudia Scholl (Functional Genomics)
DKTK: Frankfurt/Mainz, Munich, Dresden (Berlin, Essen, Freiburg)

MOLECULAR STRATIFICATION PROGRAMS
Mi-Oncoseq vs. UCSD PREDICT vs. NCT MASTER

Program	***Analyses***	***Patients***	***Actionable[1]***	***Action***	***Outcome***
Mi-Oncoseq	WES RNA-seq	369	59%	23%	Response
UCSD PREDICT	Panel-seq (236 genes)	347	–	25%	Response, PFS, OS[2]
NCT MASTER	WES RNA-seq	212	59%	18%	Response

[1]Rationale for drug in development or off-label use of approved drug; prediction of response to treatment; predisposing germline alteration

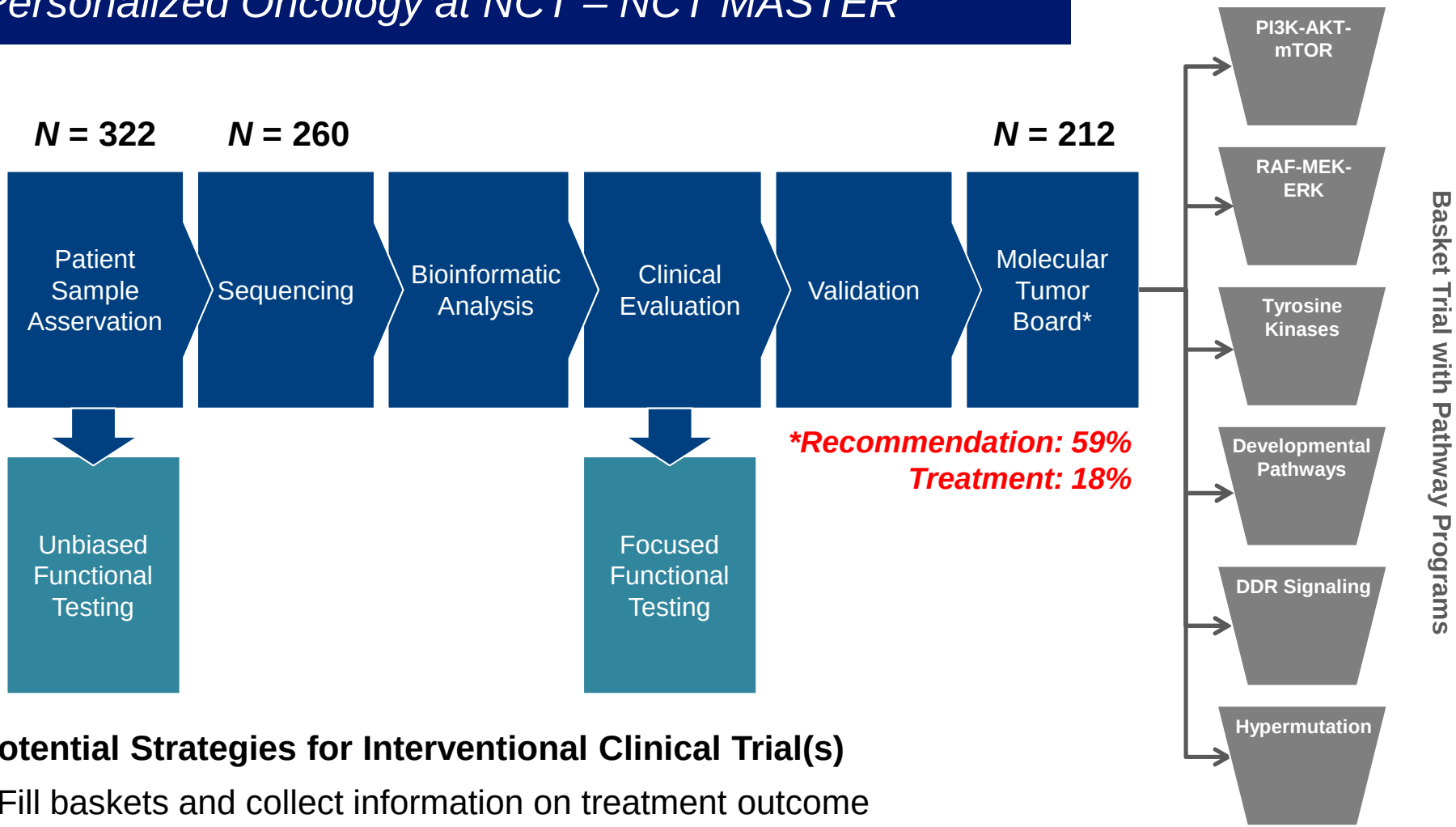
*[2]**SD/PR/CR:** 35% vs. 16% (P=0.02); **PFS:** 4 vs. 3 months (P=0.04); **PFS2/PFS1 ≥1,3:** 45% vs. 19% (P=0.004 and P=0.06); trend for superior PFS following treatment „matched directly“ vs. „matched indirectly“ (P=0.1); **OS:** 14 vs. 11 months (NS)*

LONG-TERM OUTCOME OF STRATIFIED THERAPY
Meta-Analysis of Targeted Treatment Strategies

- **UC San Diego, MD Anderson Cancer Center, Institut Gustave Roussy, WIN Consortium, ASCO**
- **570 phase 2 trials; 32,149 patients; targeted agents as monotherapy; 641 treatment arms**
- **Therapy according to biomarker vs. unselected treatment**
- **Superior outcome compared to conventional chemotherapy through targeted therapy according to biomarker ("matched directly" and "matched indirectly")**
  - Response, PFS, OS (each $P<0.0001$)
  - Toxicity ($P<0.001$)
- **Inferior outcome compared to conventional chemotherapy through unselected "targeted" therapy**
  - Response, PFS (each $P<0.0001$)
  - OS ($P=0.048$)
- **Better outcome with targeted approaches based on genomic alterations (mutations) vs. aberrant expression (RNA, protein) as biomarker**
  - Response, PFS, OS (each $P<0.05$)

Schwaederle et al. ASCO Annual Meeting 2015

NCT MOLECULAR DIAGNOSTICS PROGRAM
Personalized Oncology at NCT – NCT MASTER



Potential Strategies for Interventional Clinical Trial(s)

- Fill baskets and collect information on treatment outcome
- Identify successful baskets (OR or SD for ≥6 months in ≥2/10 patients)
- Define trial design, for example:
    - "Randomization" between patients receiving genomics-guided treatment and patients receiving standard of care (due to logistical or regulatory reasons etc.)

In collaboration with Annette Kopp-Schneider and Axel Benner, Division of Biostatistics, DKFZ

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