Individualisierte Arzneimittelentwicklung

Von Einer für Alle … zu Alle für Einen

Ugur Sahin

Mainz 25th May 2012

Forschungscampus in Mainz

TRON

Gutenberg University
- Basic & Disease orientied Immunological research since 30 years
- Biomarker research since 15 years
- Pharmaceutical research since 10 years

International Networking

Cancer Immunotherapy Consortium, 2002

Drug Developing Companies

Ganymed 2001, BioNTech 2009

Translational Center

TRON 2010

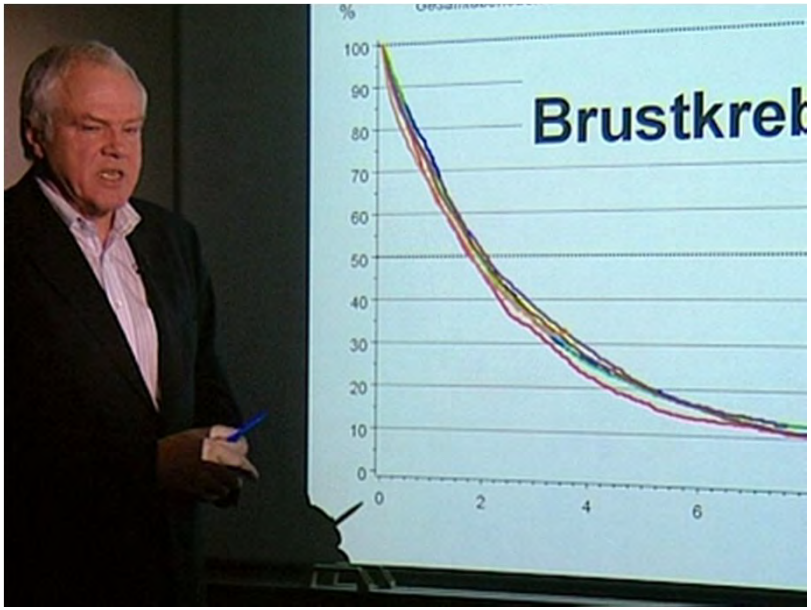
R&D Cluster

C|3 **Rhine Main Immune Intervention Cluster**
(Winner German Spitzencluster Competition 2012
(40 Mio € funding by BMBF)

Daten aus Krebsregistern

„Wenn in diesen 25 Jahren große Fortschritte erreicht worden wären, müssten dies über die Daten des Tumorregister sehen..

..Wir sehen hier nur eine geringfügige Verbesserung, die in der Größenordnung von drei Monaten liegt.“

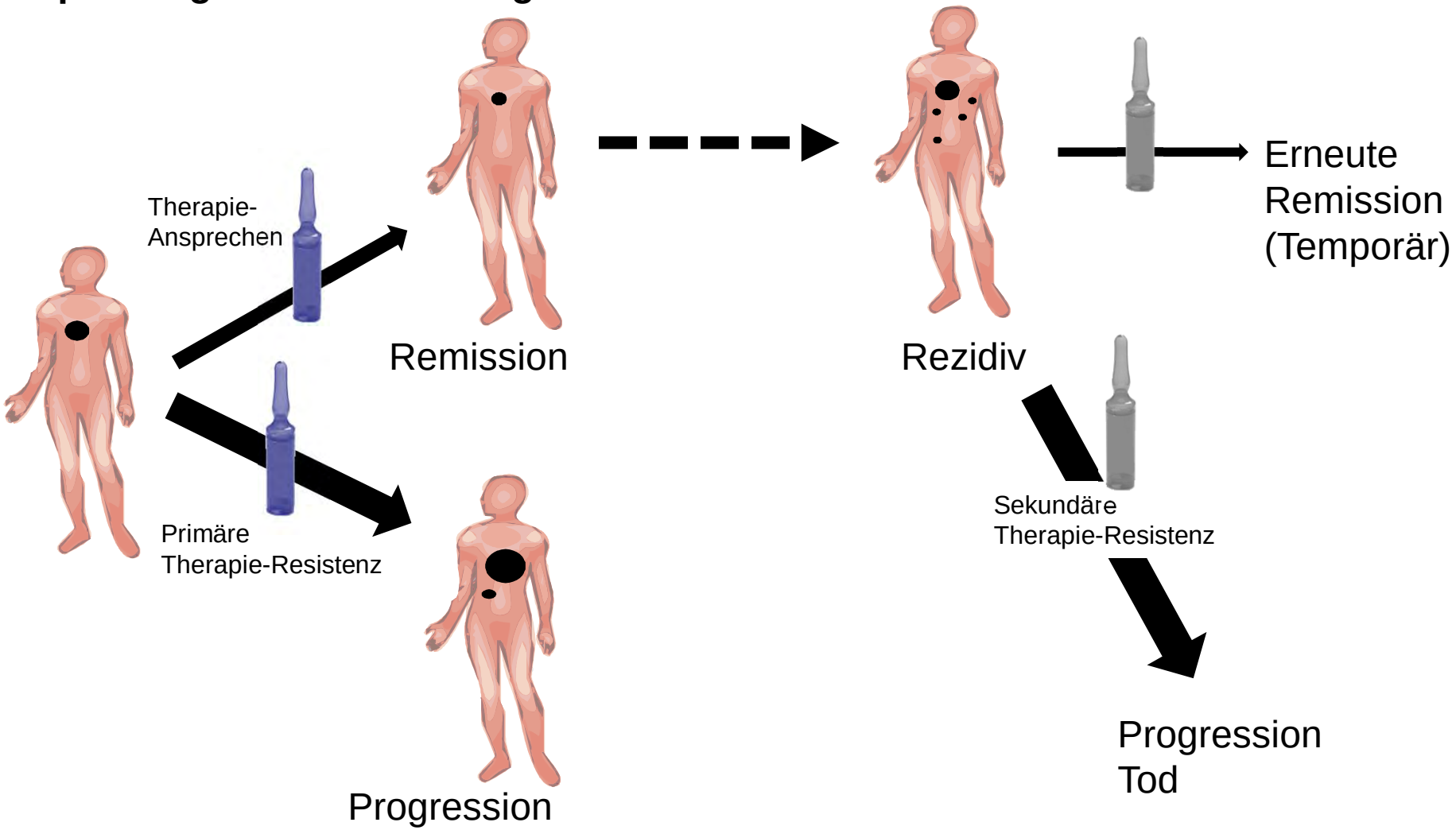


Prof. Dieter Hölzel, Tumorregister München, Interview SWR, 2008

Verbesserung der Prognose von Krebserkrankungen ist eine Herausforderung an die Gesellschaft

Das klassische Modell - Ein Wirkstoff für Alle

Bsp.: Fortgeschrittener Lungenkrebs



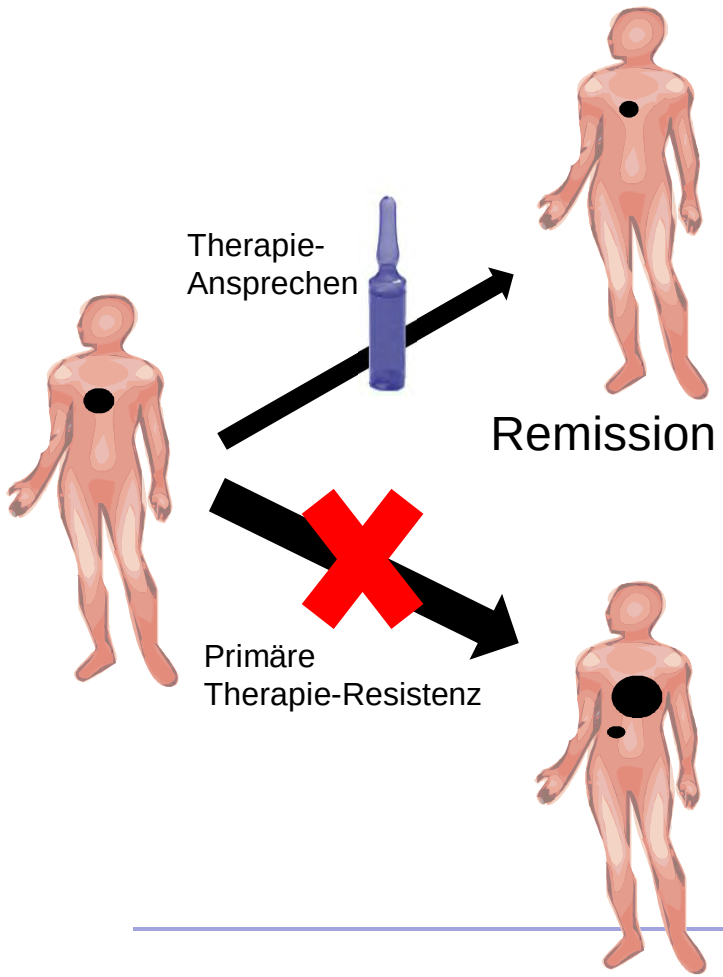
Erstlinientherapie

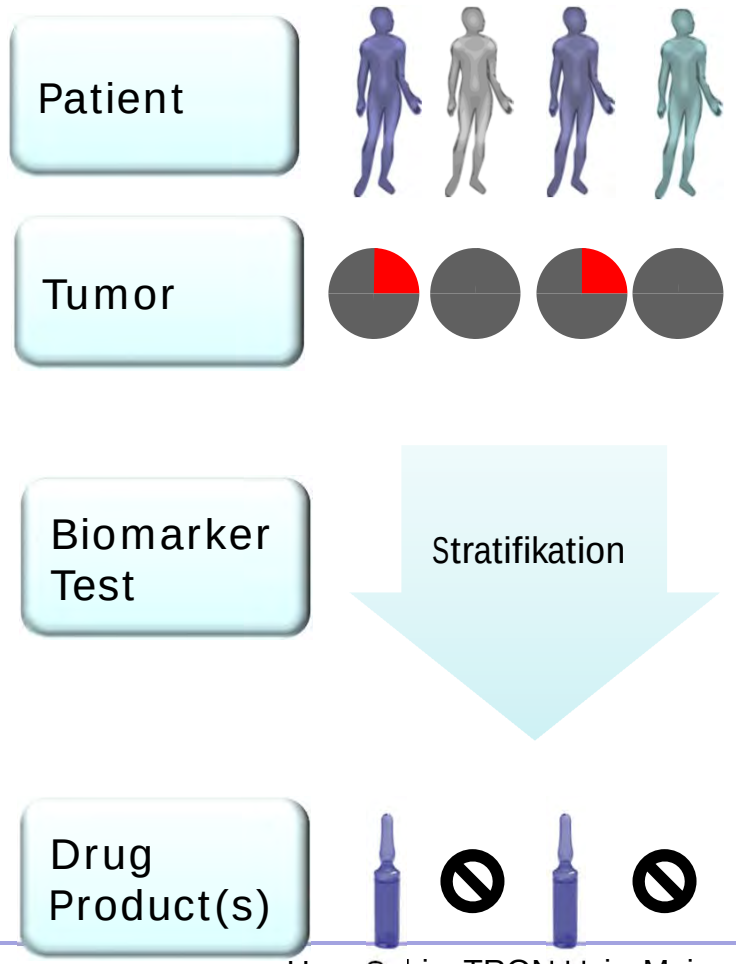
Zweitlinientherapie

Ein Wirkstoff, …. nicht mehr für Alle...

Ziel: Nichtbehandlung von primär Therapie-resistenten Patienten (Erhöhung Ansprechrate, Vermeidung unnötiger Therapie)

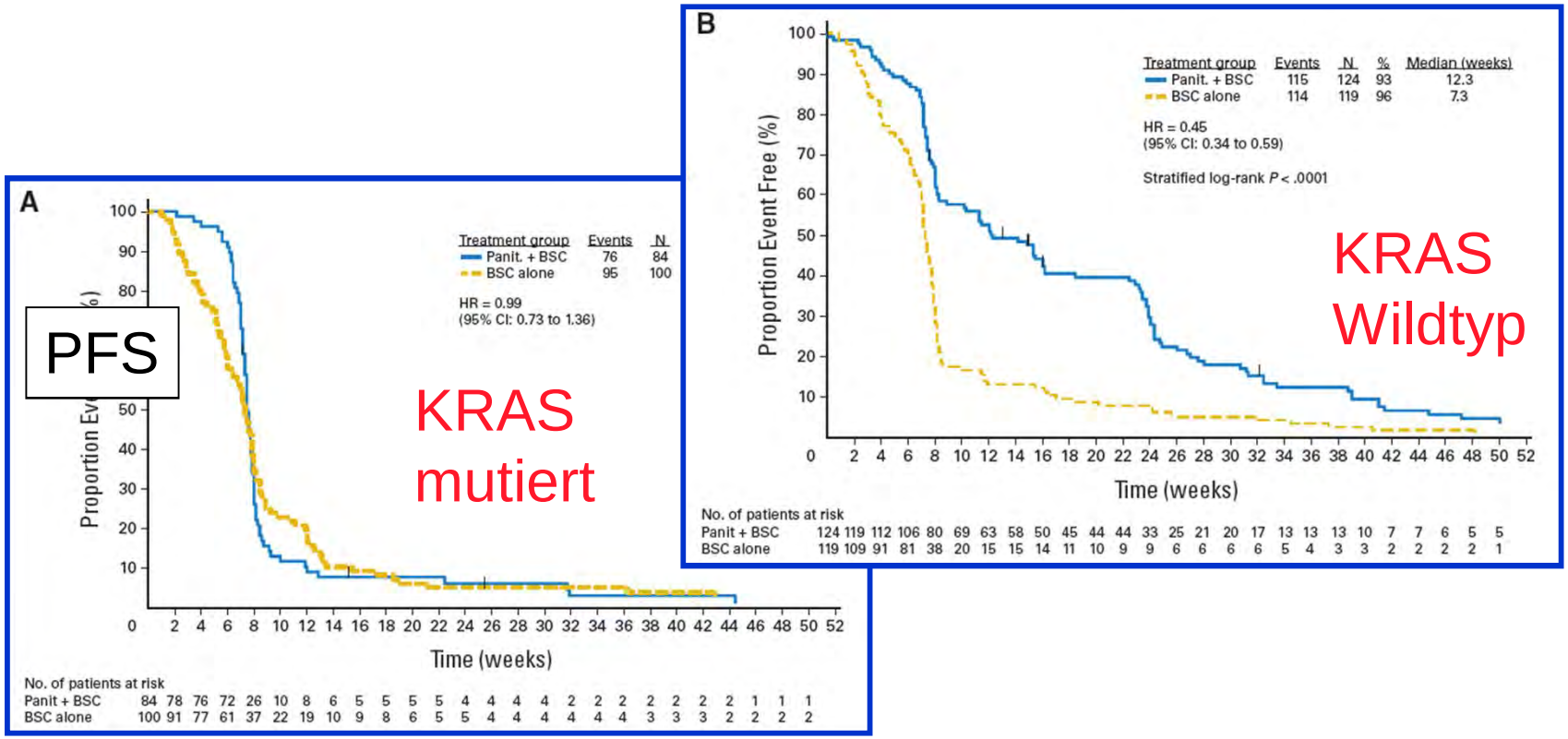
Vorgehen: Ein-/Ausschluss über Tests für <u>empirisch identifizierte</u> Biomarker





Bsp. KRAS Mutation bei EGFR Antikörpertherapie

Panitumumab bei kolorektalen Tumoren



Phase III Studie Panitumumab (anti-EGFR) + Best Supportive Care vs BSC in Patienten mit Chemotherapie-refraktären metastasierenden Kolorektalen Tumoren

➔ Zulassung von Vectibix beschränkt auf Patienten mit KRAS Wildtyp

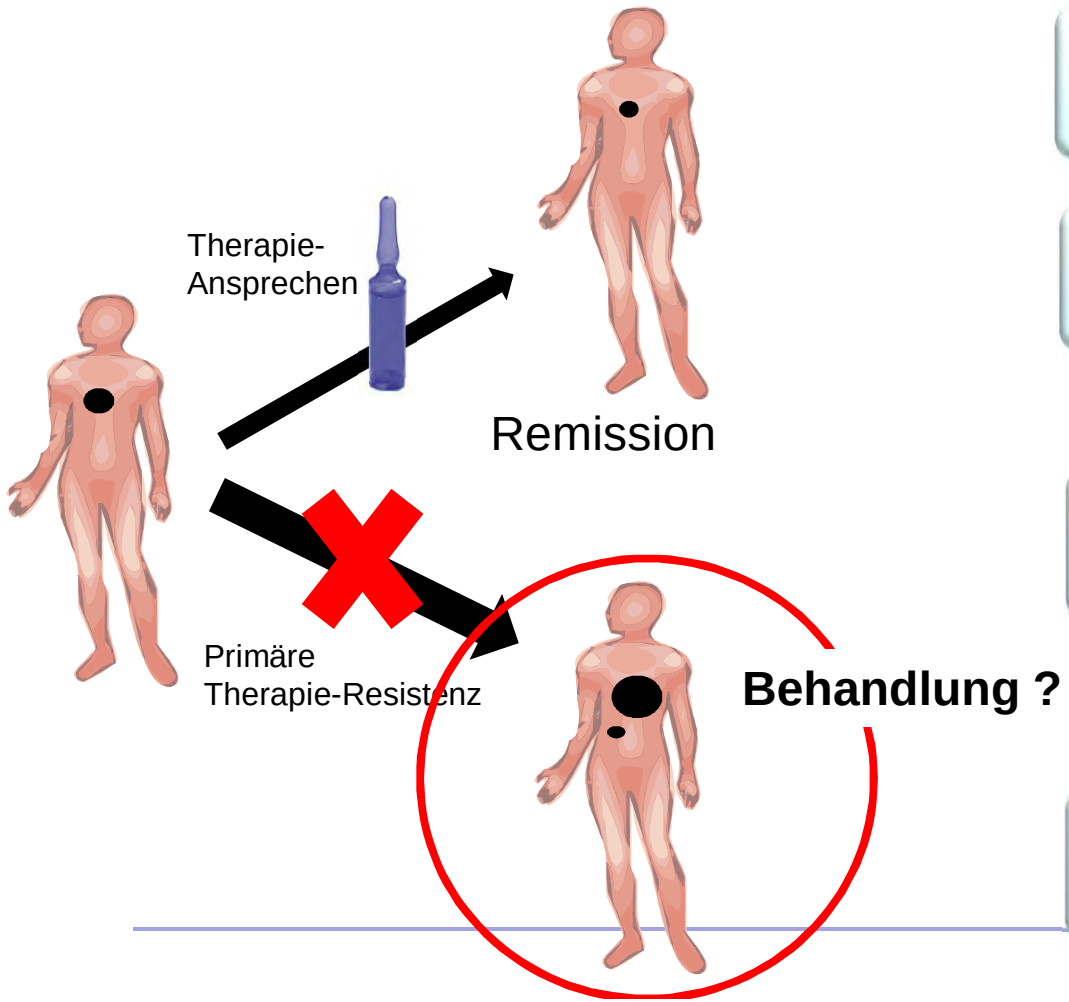
Amado et al., JCO März 2008

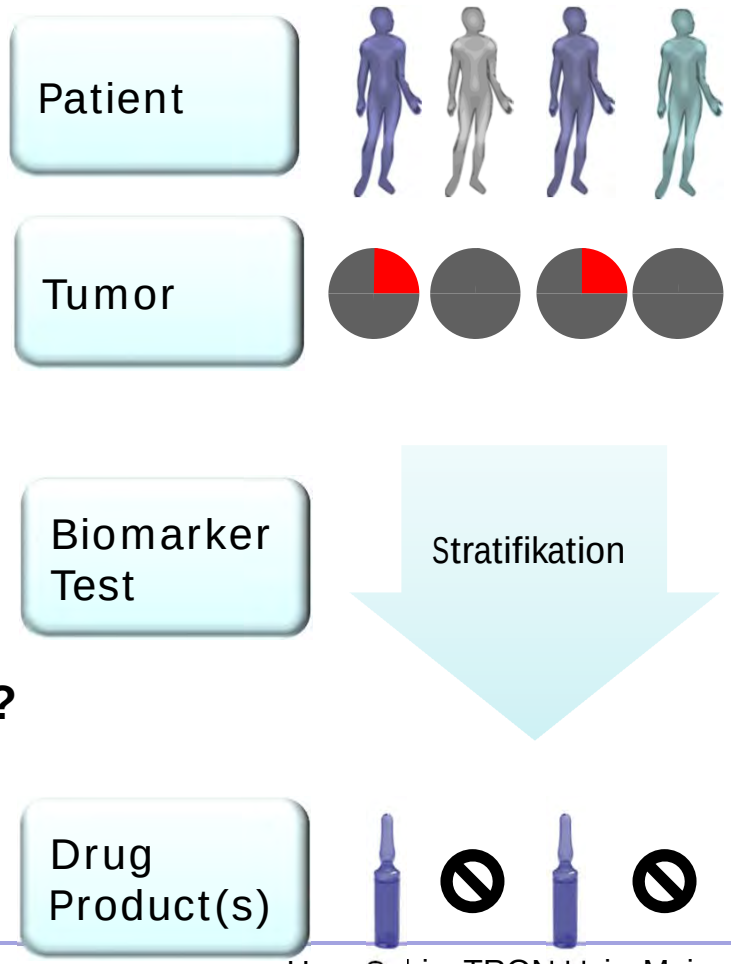
Strategie 1 – Ausschluss von Non-Respondern

Ein Wirkstoff, nicht mehr für Alle...

Ziel: Nichtbehandlung von primär Therapie-resistenten Patienten (Erhöhung Ansprechrate, Vermeidung unnötiger Therapie)

Vorgehen: Ein-/Ausschluss über Tests für <u>empirisch identifizierte</u> Biomarker





Neue gezielte Wirkstoffe… für Einige

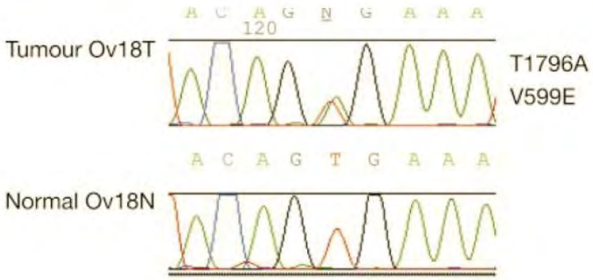
1. Cancer Genome Projekt

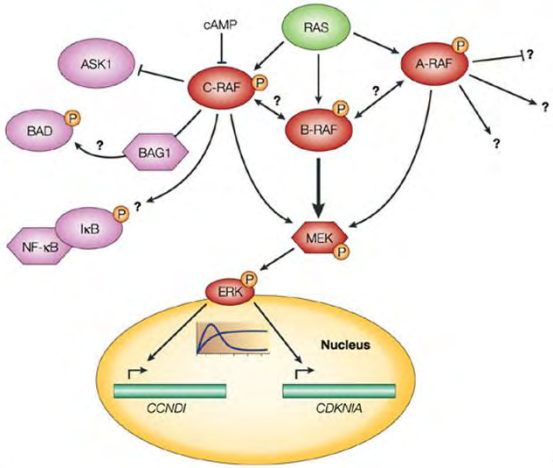
- Initiiert durch Mike Stratton in 2000
- Finanziert durch Welcome Trust & EUCIP
- Sequenzierung von Exons aus Zellinien
- Suche nach Mutationen bevorzugt Targets, die Druggable sind

Mike Stratton
Director Welcome Trust Sanger Institute

Mutations of the *BRAF* gene in human cancer

NATURE | VOL 417 | 27 JUNE 2002 |





60% Mutationen in Malignem Melanom
7% übrige Krebsarten

.. 8 Jahre später

Inhibition of Mutated, Activated BRAF in Metastatic Melanoma

Keith T. Flaherty, M.D., Igor Puzanov, M.D., Kevin B. Kim, M.D., Antoni Ribas, M.D.,
Grant A. McArthur, M.B., B.S., Ph.D., Jeffrey A. Sosman, M.D., Peter J. O'Dwyer, M.D., Richard J. Lee, M.D., Ph.D.,
Joseph F. Grippo, Ph.D., Keith Nolop, M.D., and Paul B. Chapman, M.D.

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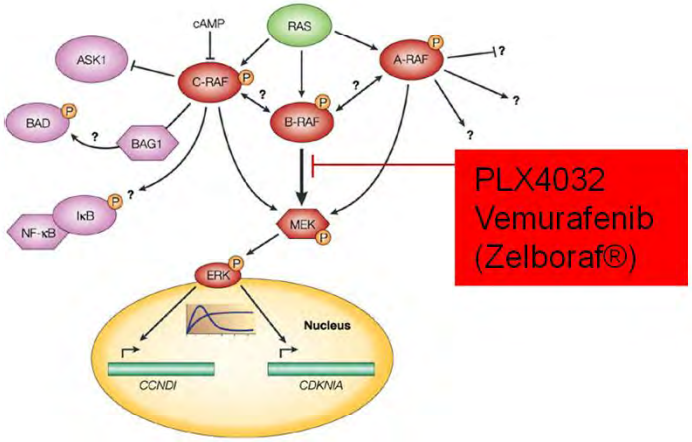

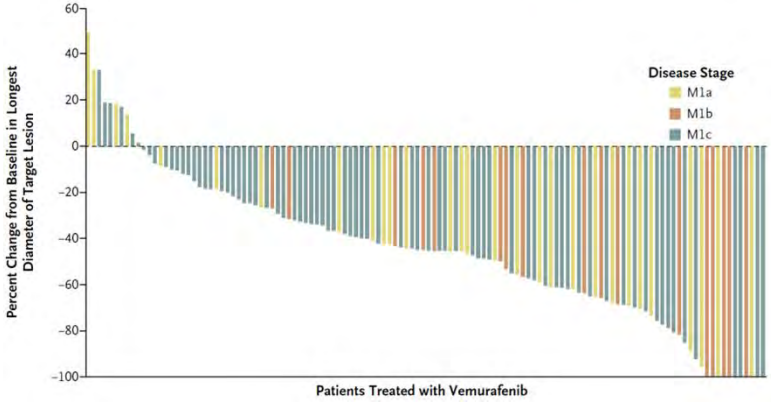

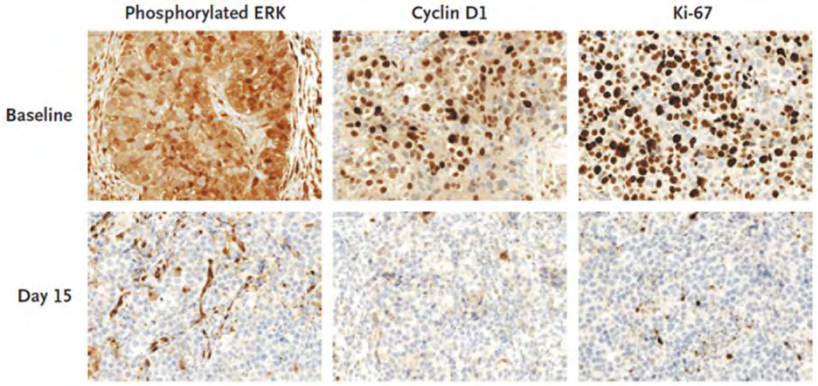

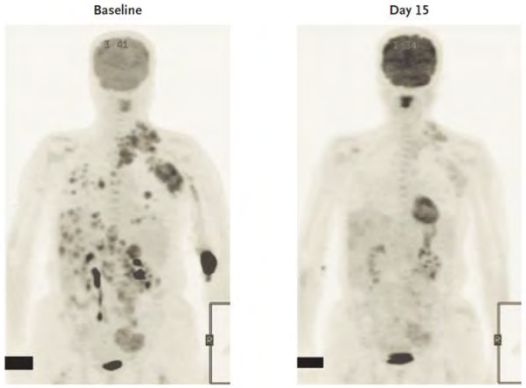

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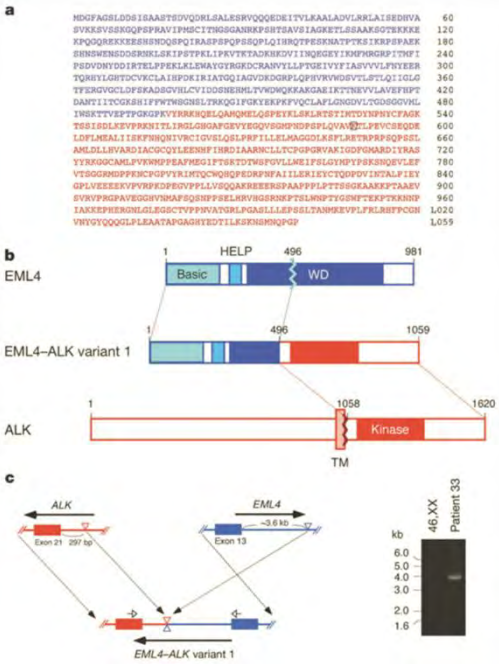
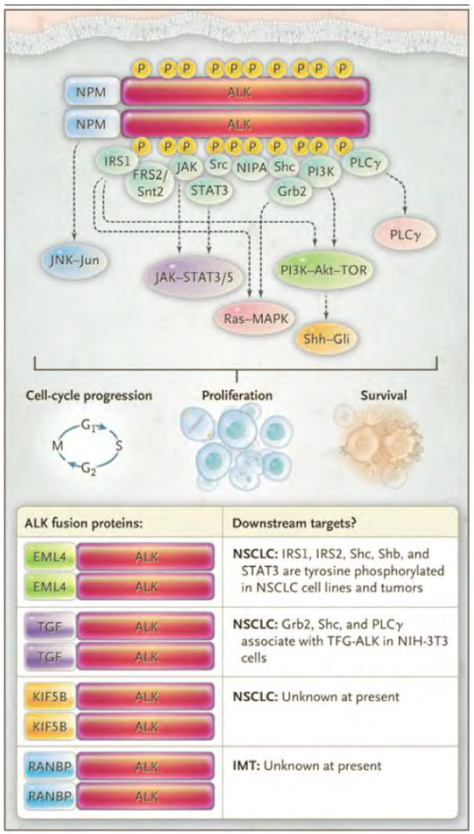
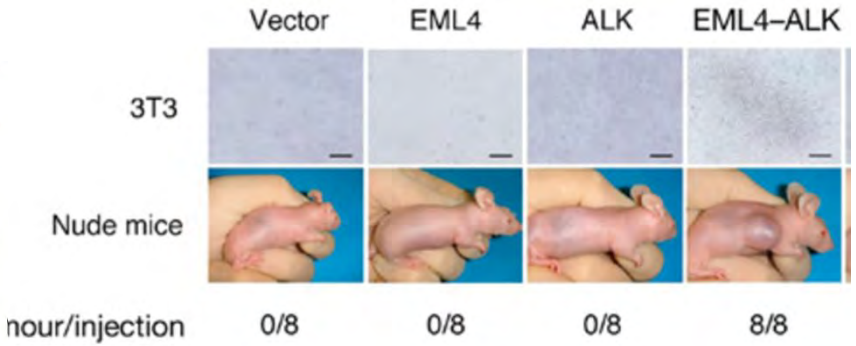
Bsp.: EML4-ALK Fusionsprotein bei NSCLC

EML4-ALK Fusionsprotein

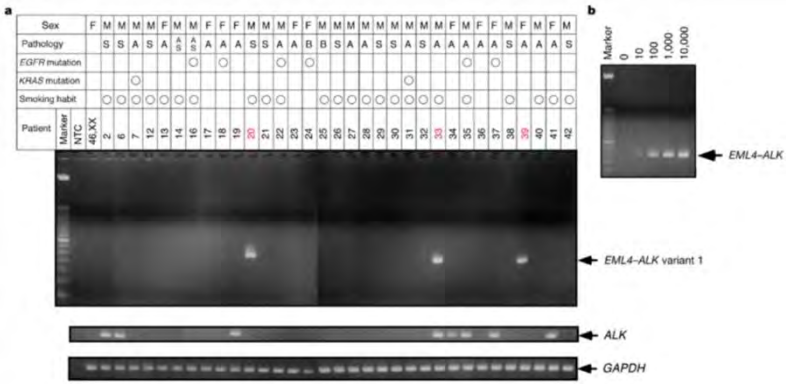




Transformation

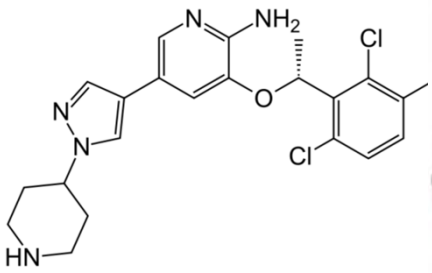


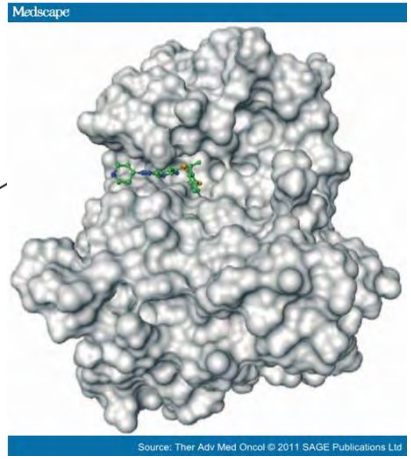
4-7% Prävalenz im NSCLC



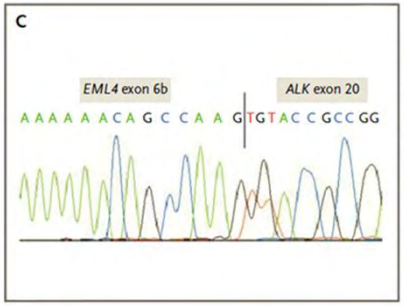
Soda et al, 2007 nature

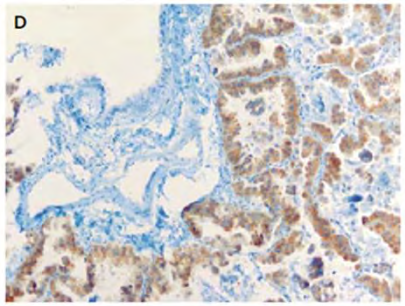
.. Drei Jahre später

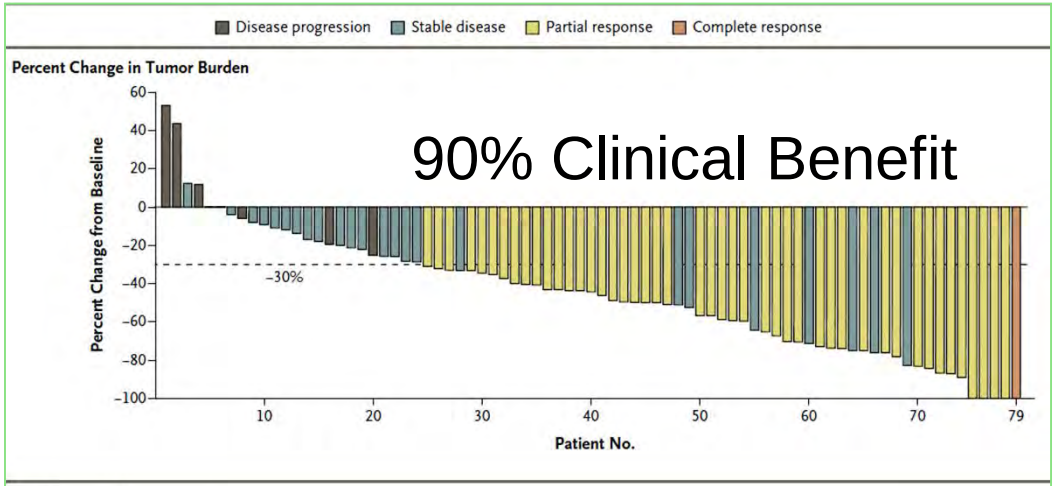


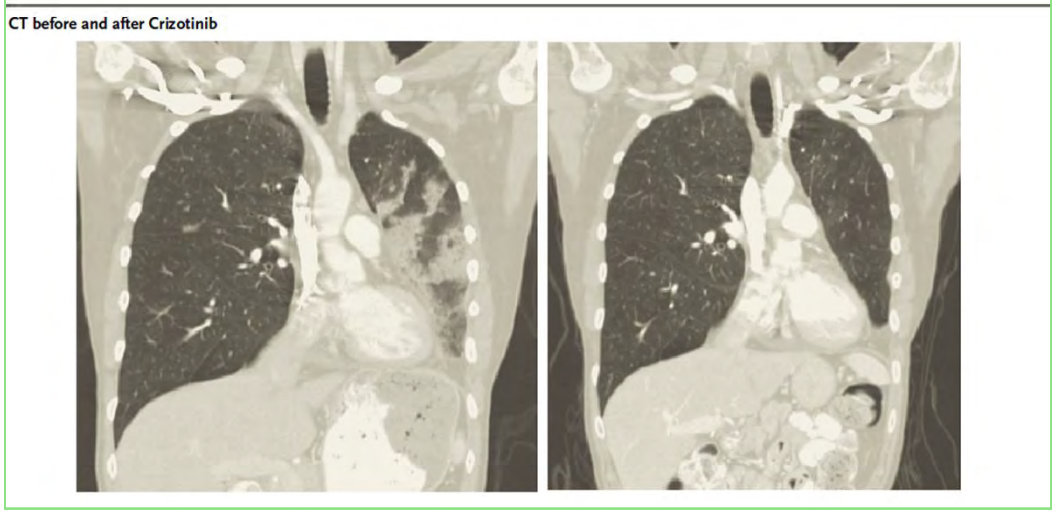


Crizotinib- Protein Kinase Inhibitor









Anaplastic Lymphoma Kinase Inhibition in Non–Small-Cell Lung Cancer

The NEW ENGLAND JOURNAL of MEDICINE

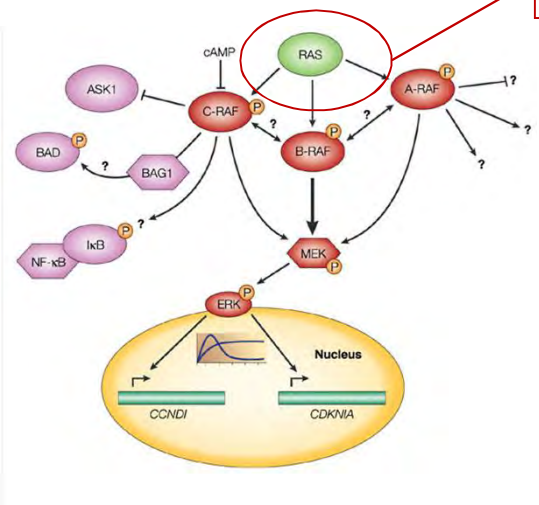
2010

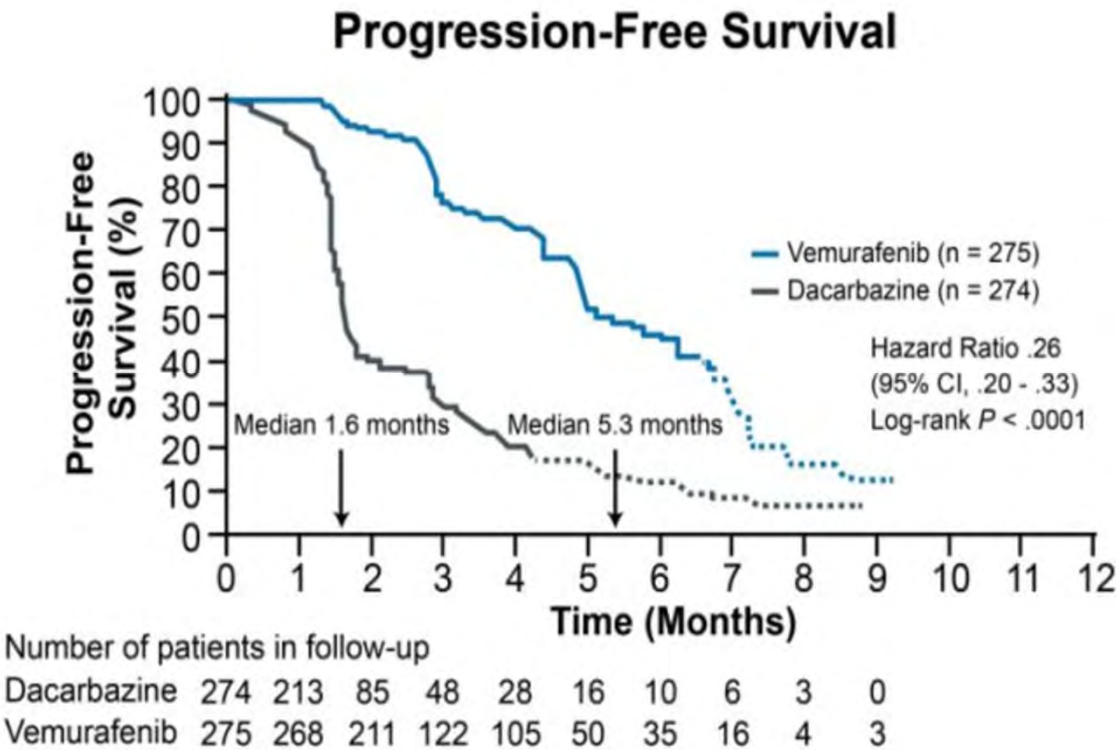
Vemurafenib - Temporärer Benefit

Langzeitüberleben limitiert durch Resistenzen

Melanomas acquire resistance to B-RAF(V600E) inhibition by RTK or N-RAS upregulation

nature

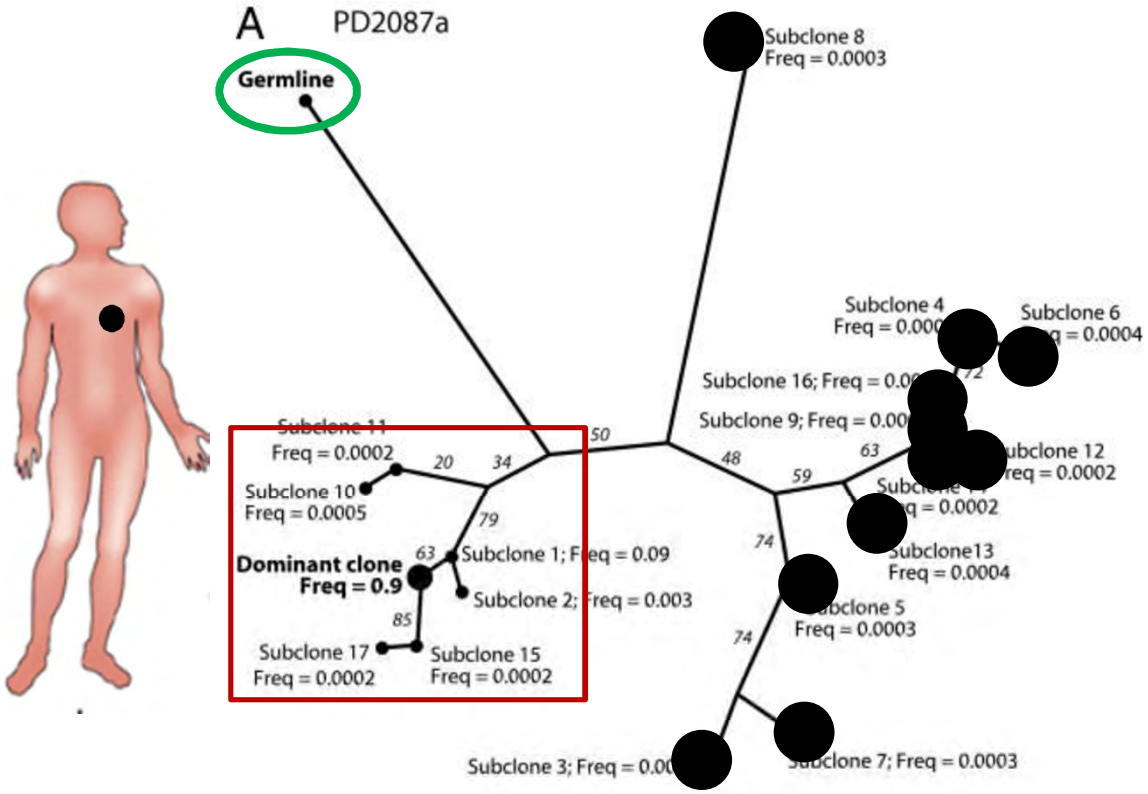




Nature **468**, 973–977 (16 December 2010)

Molekulare Heterognität – Ursache für Resistenz

Individuelle Klonale Heterogenität



Subclonal phylogenetic structures in cancer revealed by ultra-deep sequencing

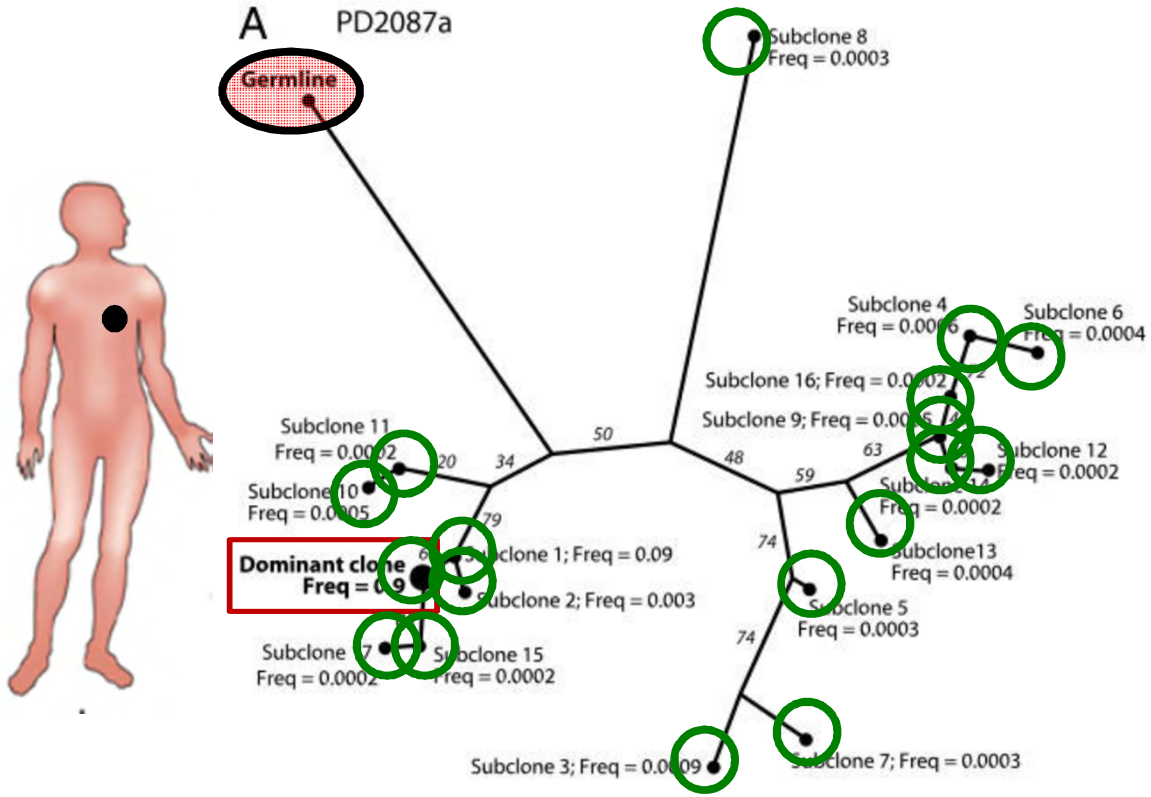
Peter J. Campbell*, Erin D. Pleasance*, Philip J. Stephens*, Ed Dicks*, Richard Rance*, Ian Goodhead*, George A. Follows†, Anthony R. Green†, P. Andy Futreal*†, and Michael R. Stratton*‡§

Behandlung kontrolliert
Wenige Klone des Tumors

Primär und sekundär resistente Klone Wachsen auf

Lösungsansatz – Molekulare Heterogenität als Target

Individuelle und Klonale Heterogenität



Subclonal phylogenetic structures in cancer revealed by ultra-deep sequencing

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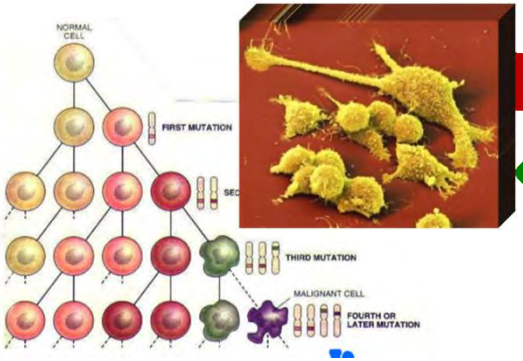
Individuell auf das Mutationsprofil des jeweiligen Tumors maßgeschneiderte **Polyspezifische Immuntherapie**

Krebs - Mehrdimensionale Komplexitität

TRON

Krebszellen



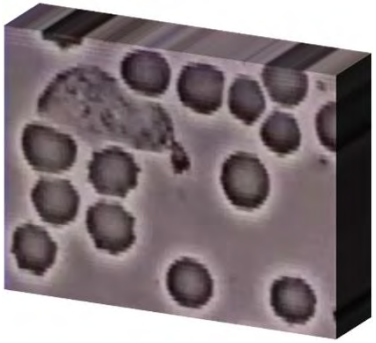
Therapeutische Immunomodulation

Immunosuppression

Cancer Recognition / Immunoediting

Antigen Specifische Immuntherapie

Immunsystem



Klonales Immun- Repertoire
- T Zellen, Antikörper

Genetische/ Epigenetische Mutationen
> 100 Mutationen/Krebs,> 95% Individuelle Mutationen
> Individuelle Spektrum von Krebsgenen / Transkripten
➔ Tumorwachstum, Resistenz, Metastasierung, Rezidive
➔ Immunosuppression

Keimbahn Variation

- HLA-Antigens
- Genetic Polymorphismen

Krebspatienten

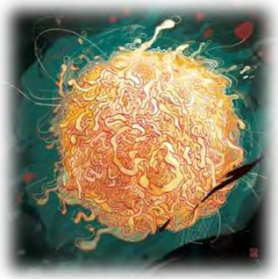
Individueller Patient - Reduzierte Komplexität

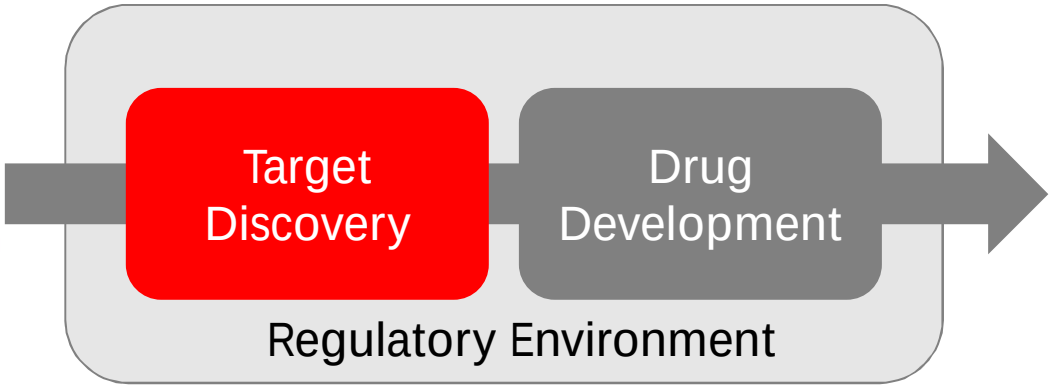
Individueller Patient

Benötigte Information:

- HLA Haplotype
- Shared Cancer Antigen Profile
- Individuelles Mutations Profile

Patient Tumor





Patient Therapie

..Forschungsstrategie

- Komplettes Abbild von Immuntherapeutisch nutzbaren tumorselektiven Zielstrukturen

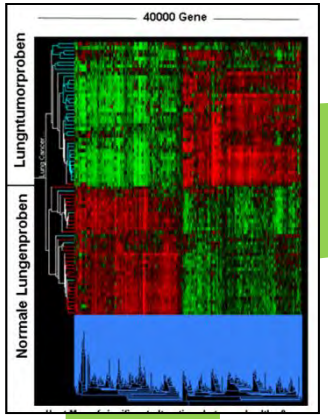
- Immuntherapie- Plattformen, die multiple Zielstrukturen effizient in potente Leitstrukturen übersetzen können

- GMP taugliche Prozesse, die geeignet sind für die individualisierte Herstellung von Arzneimitteln

20 Jahre Suche nach Idealen Zielstrukturen...

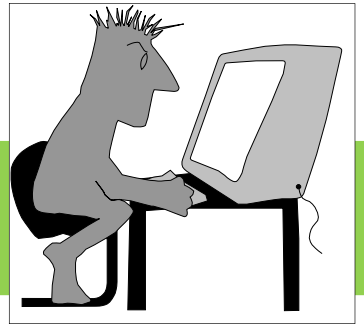
TRON

..From Genes to HLA Epitopes



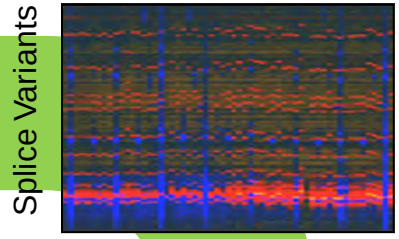
Microarray Bodmaps[#3]

.. Towards a Profiler Approach



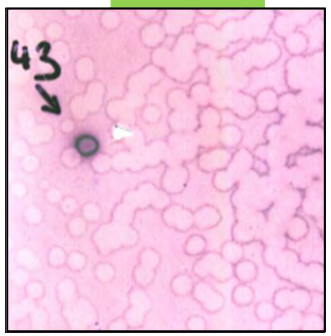
„In silico Cloning“

.. Focus on Splice Variants



← Tissues →

Splice Display



SEREX[#1,2]

..Spontanous Immunorecognition of Cancer

Zielstrukturen, die …

..prävalent in Krebs

..aber nicht im Gesunden sind

.. Towards a Complete Picture

Next-GenerationSequencing

#1Sahin et al, PNAS 1995, #2Türeci et al, PNAS 1998, #3Sturniolo et al, Nat. Biotech 1999, #4, #5Koslowski et al, Cancer Res. 2001, 2008

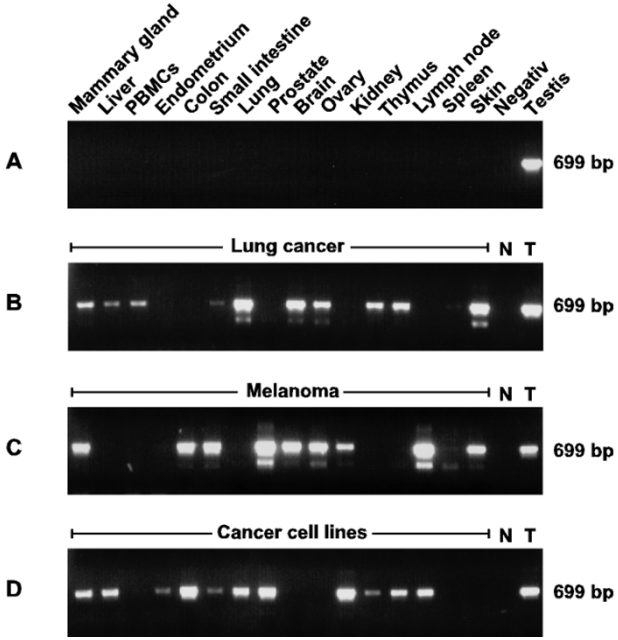
Tumor Selektive Antigene – Shared Tumor Antigens

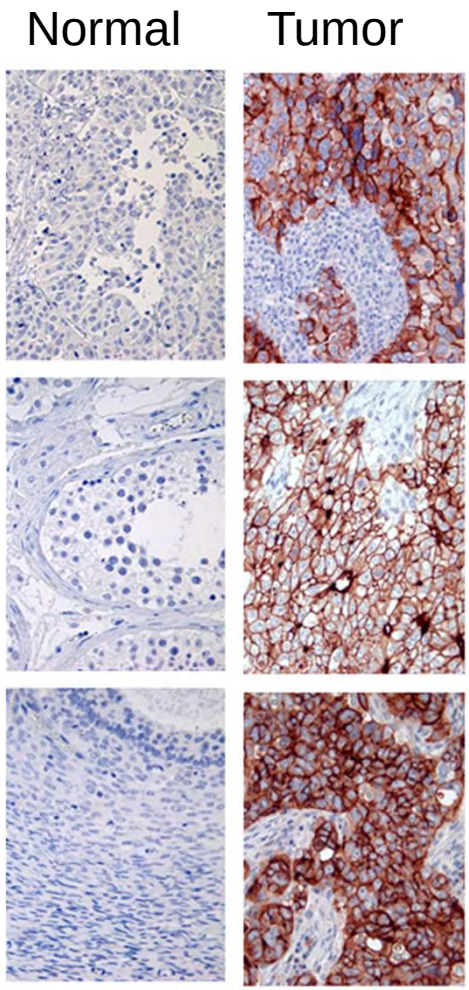
Multiple Gene/ Genfamilien

Nicht exprimiert in Toxizitäts-relevanten Normalgeweben

Aktivierung in in einer Vielzahl von Tumoren

Spontane / Induzierbare Immunogenität

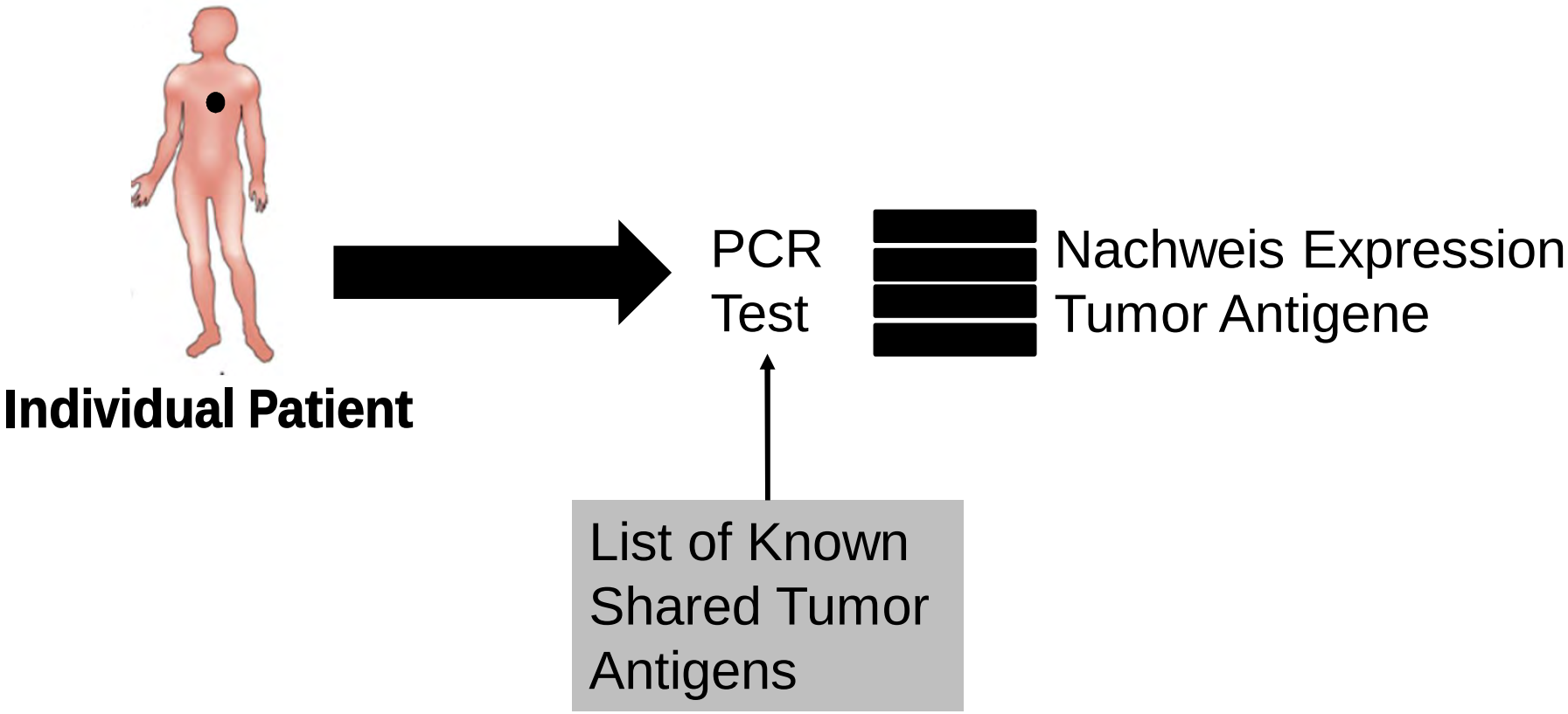




> 50 Gene/ Genfamilien
8 identifiziert mit T-Zellklonierung
>20 identifiziert durch SEREX
>50 identifiziert durch Genomics/Bioinformatik

Van der Bruggen et al., 1991 , Sahin et al., 1995, 1997, Tureci et al., 1997

Individuelle Signaturen für Shared Tumor Antigens



Letter to the Editor

Expression of multiple cancer/testis (CT) antigens in breast cancer and melanoma: Basis for polyvalent CT vaccine strategies

Analysis of Individual tumors for tumor antigen expression

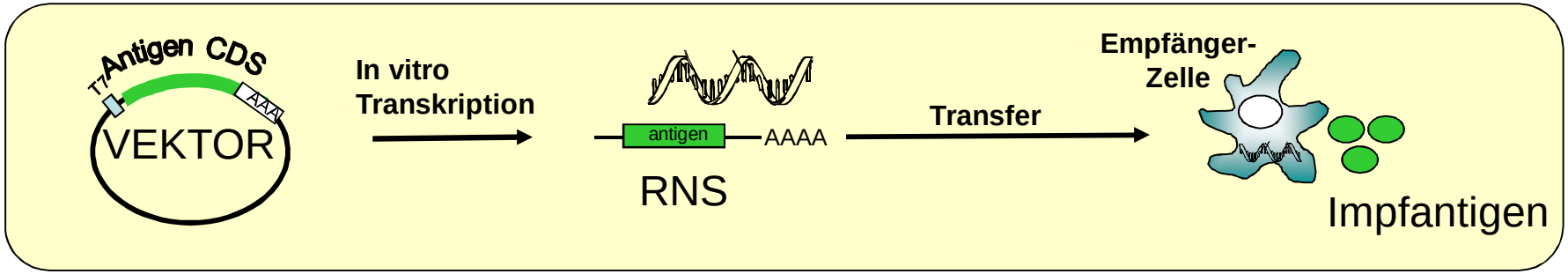
TABLE II – EXPRESSION OF CT-ANTIGENS IN MALIGNANT MELANOMA[1]

	17/23 (74%)	SSX-1	SSX-2	SSX-4	SCP-1	NY-EsoI	MAGE-1	MAGE-3	GAGE	BAGE
Antigen Rich	Mm 1	+	+	+	+	+	+	+	+	+
	Mm 2	−	−	−	−	+	+	+	−	+
	Mm 3	−	−	−	−	+	−	−	−	−
	Mm 4	−	−	−	−	+	−	+	−	−
	Mm 5	−	−	−	−	+	−	+	+	−
	Mm 6	−	+	−	−	+	+	+	−	+
Antigen Poor	Mm 7	−	+	−	−	−	−	−	−	−
	Mm 8	−	+	+	+	+	+	+	−	−
	Mm 9	−	−	−	−	−	−	−	−	+
	Mm 10	+	+	+	−	−	+	+	−	+
	Mm 11	+	+	−	−	−	−	−	+	−
	Mm 12	+	+	+	−	−	+	+	−	−
	Mm 13	+	+	+	−	−	−	−	−	−
	Mm 14	+	−	−	−	−	+	+	−	−
	Mm 15	−	+	+	−	+	−	+	+	−
	Mm 16	+	+	−	−	+	+	+	−	−
	Mm 17	−	−	−	−	+	−	+	−	−
	Number	7	10	6	2	10	8	12	4	5
	(%)	(30)	(44)	(26)	(8)	(44)	(35)	(52)	(17)	(22)

Sahin et al., Int. J. Cancer 1998

RNS als Wirkstoffklasse für Impfstoffentwicklung

Ausgangsbasis: Verfügbarkeit von molekular definierten Tumorantigenen
Suche nach Impfstoffverfahren für klinische Translation



Rationale Gründe für RNS

- Einsetzbar unabhängig vom HLA-Status
- Induktion von kombinierten Immunantworten (CD4, CD8 T-Zellen und Antikörper)
- Keine Integration in das Genom (nicht mutagen)
- Nutzbarkeit verschiedener genetischer Werkzeuge
- Produktion und Reinigung unkompliziert
- GMP-Herstellung sehr schnell und kostengünstig

Technisches Problem Sehr kurze HWZ durch schnellen Abbau durch Ribonukleasen

BioNTech / Ribological Technologie-Plattform

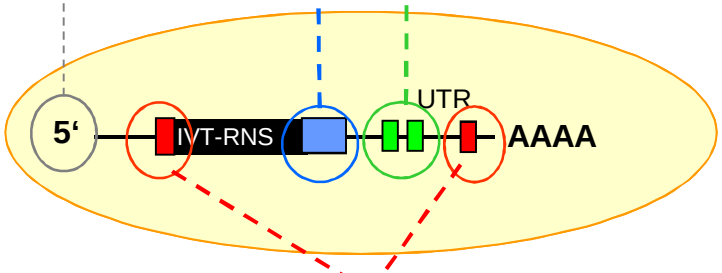
TRON

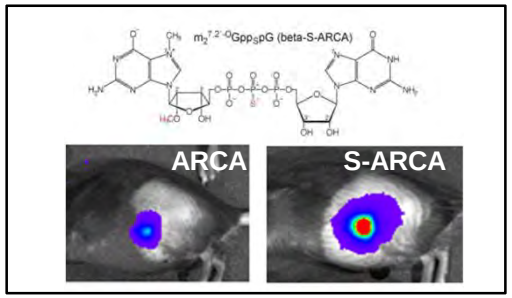
Modifizierte Cap-Nukleotide zur Inhibition des Abbaus

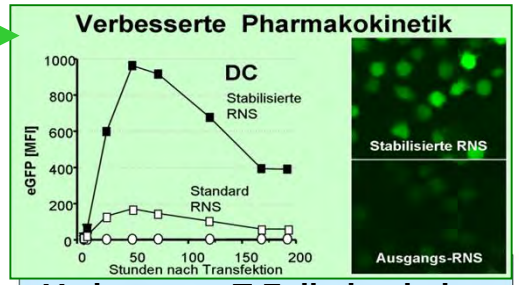
Reg. Sequenzen zur Stabilisierung und verbesserten RNS Translation

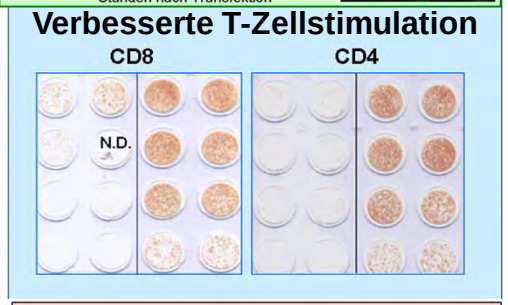
HLA-Signalsequenzen zur Verbesserung Antigenpräsentation

Elemente, die an Zytokin- und Toll-Like Rezeptoren aktivieren

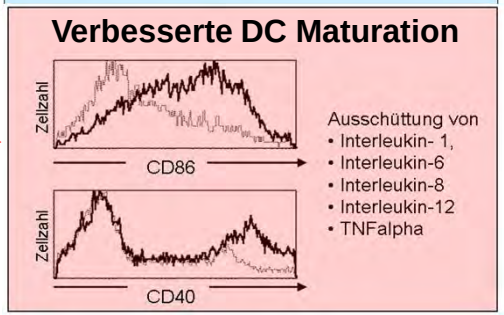








x 5000
Potenz ↑



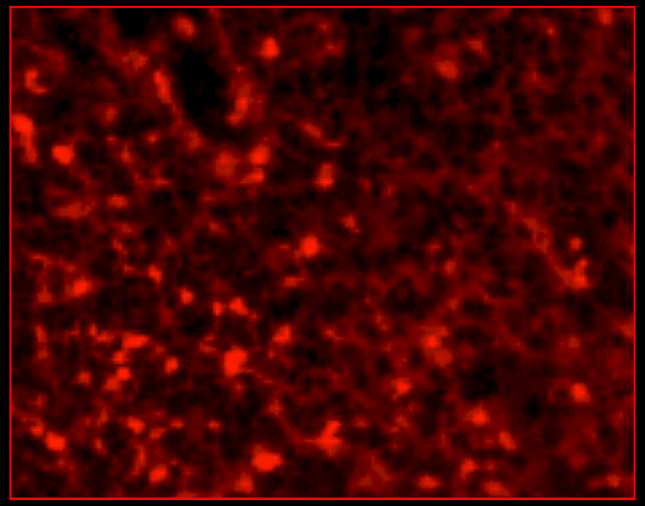
Adjuvanz-
Funktion

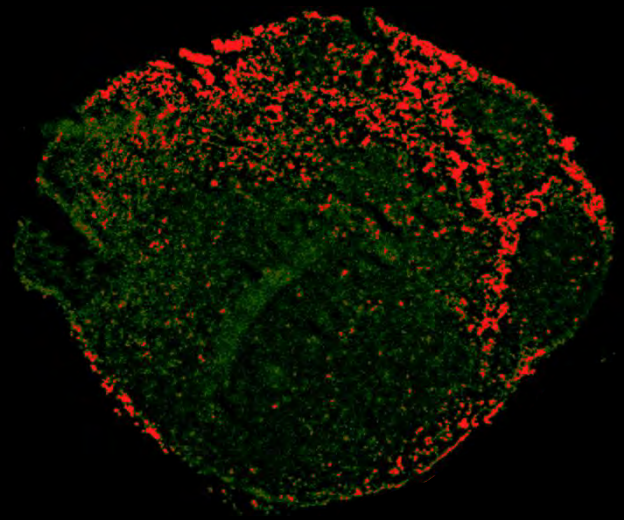
Kreiter et al, Current Opinion Immunology, 2011*

Targeting of RNA into Lymph Node DCs

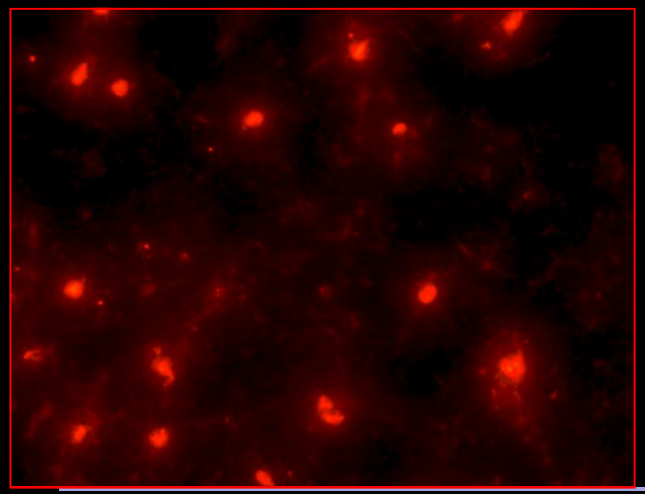
Analysis of Biodistribution after Injection of RNA-CY3

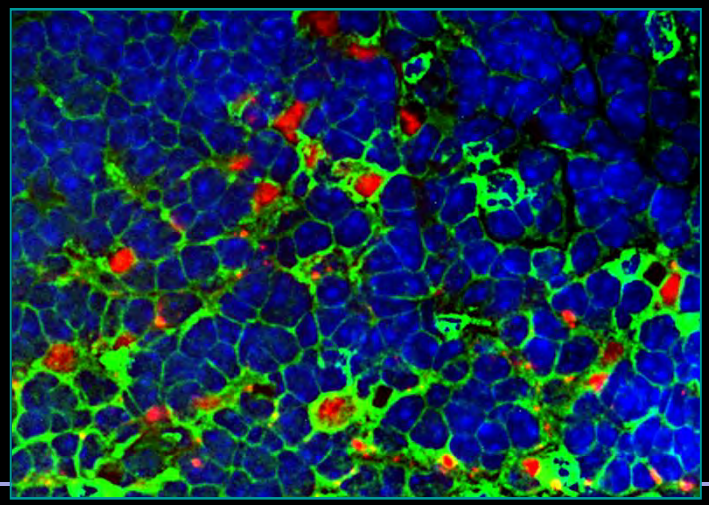
5min





30min





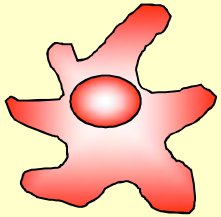
DAPI
RNA-CY3
CD11b

Stand der Technik und Innovation

TRON

Stand der Technik

Transfektion
In vitro



Adoptiver
Transfer

Klassische RNS-Impfstoffe

- Ungünstige PK
- Geringe Potenz
- Geringe Adjuvansfunktion

Dendritische Zellen

- Gute Antigenpräsentation
- Gute Adjuvansfunktion

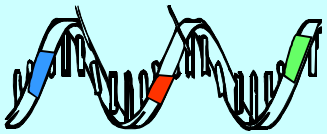
* Boczkowski et al., 1996; **Tendeloo et al., 2002, ***Pan et al., 2005; ***Heiser et al., 2002, Hoerr et al, 2000

FuE

Genet. Modifikationen
- Erhöhung der HWZ
- Opt. Immunogenität
- Opt. Adjuvanswirkung

Protektion vor RNSasen

Innovation



Riboimpfstoffe & Riboadjuvantien

Für direkte Applikation

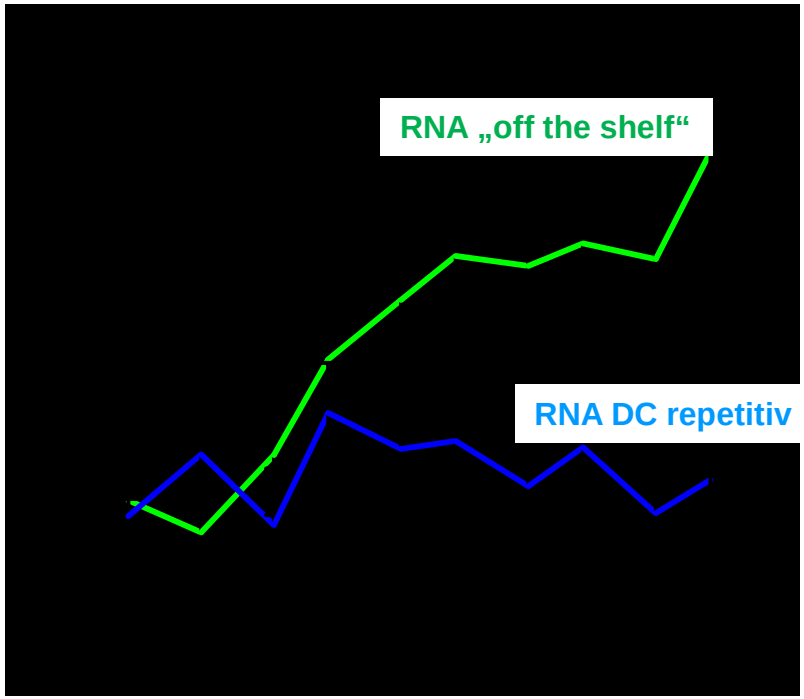
Immunoribopharmaka

- Verbesserte PK
- Hohe Potenz
- Inherente Adjuvansfunktion

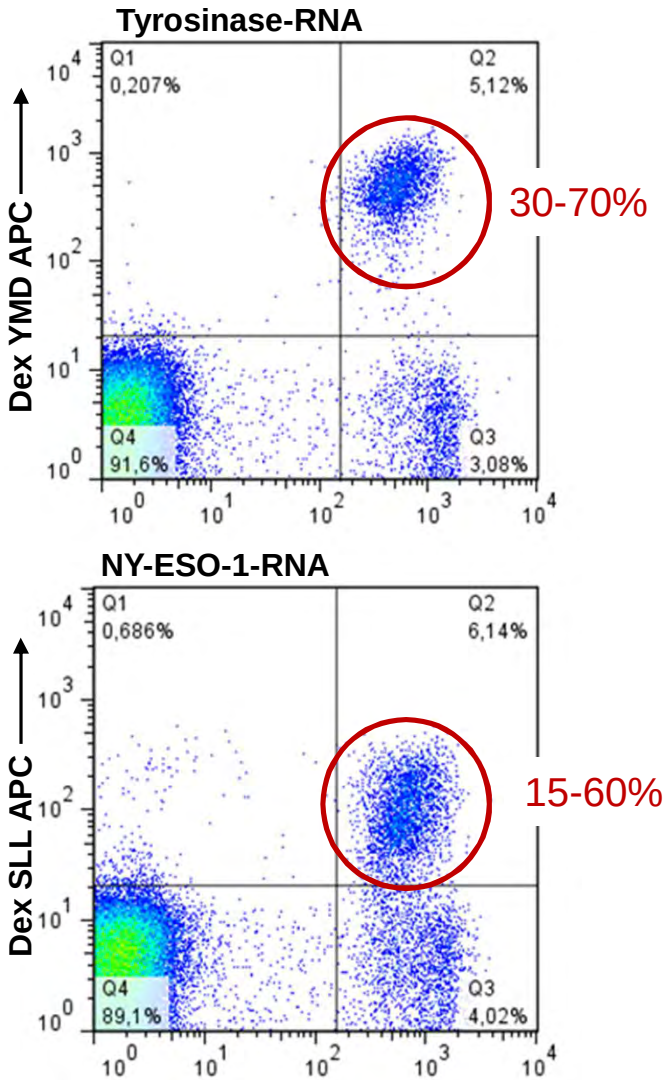
Prevention & Therapie
Krebs- u. Infektionskrankheiten

Versatil, Potente Impfplattform

Repetitive Immunisation in B6/C57L



Immune Response in A2/DR1 Mice



Mutationen als T Zell Targets

TRON

A p16^{INK4a}-Insensitive CDK4 Mutant Targeted by Cytolytic T Lymphocytes in a Human Melanoma

Thomas Wölfel,* Martina Hauer, Jörg Schneider, Manuel Serrano, Catherine Wölfel, Eva Klehmann-Hieb, Etienne De Plaen, Thomas Hankeln, Karl-Hermann Meyer zum Büschenfelde, David Beach

SCIENCE • VOL. 269 • 1 SEPTEMBER 1995

Cloning Genes Encoding MHC Class II–Restricted Antigens: Mutated CDC27 as a Tumor Antigen

Rong-Fu Wang,* Xiang Wang, Alicia C. Atwood, Suzanne L. Topalian, Steven A. Rosenberg

SCIENCE VOL 284 21 MAY 1999

A Point Mutation in the *α-Actinin-4* Gene Generates an Antigenic Peptide Recognized by Autologous Cytolytic T Lymphocytes on a Human Lung Carcinoma[1]

Hamid Echchakir,[2] Fathia Mami-Chouaib,[2,3] Isabelle Vergnon, Jean-François Baurain, Vaios Karanikas, Salem Chouaib, and Pierre G. Coulie

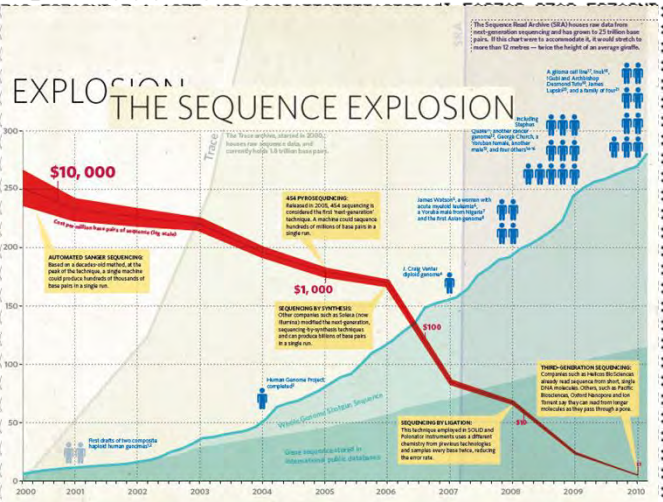
Cancer Res 2001;61:4078-4083.

The response of autologous T cells to a human melanoma is dominated by mutated neoantigens

Volker Lennerz[†], Martina Fatho[†], Chiara Gentilini[‡], Roy A. Frye[§], Alexander Lifke[†], Dorothea Ferel[†], Catherine Wölfel[†], Christoph Huber[†], and Thomas Wölfel[†¶]

PNAS | November 1, 2005 |

Erstes humanes Genom: $3 Milliarden 10 Jahre 20 Labors, Exome Resequenzierung im TRON : €3K, 3 Tage



Journal of Pathology
J Pathol 2010; **220**: 307–315
Published online 17 November 2009 in Wiley InterScience
(www.interscience.wiley.com) **DOI:** 10.1002/path.2636

Invited Review

Does massively parallel DNA resequencing signify the end of histopathology as we know it?

Samuel AJR Aparicio[1,2,3]* and David G Huntsman[2,3]

[1] Molecular Oncology, BC Cancer Agency, 675 W10th Avenue, Vancouver V5Z 1L3, Canada
[2] Centre for Translational and Applied Genomics, BC Cancer Agency, Vancouver V5Z 1L3, Canada
[3] Department of Pathology, University of British Columbia, G227-2211 Wesbrook Mall, Vancouver, BC V6T 2B5 Canada

*Correspondence to: Samuel AJR Aparicio, Molecular Oncology, BC Cancer Agency, 675 W10th Avenue, Vancouver V5Z 1L3, Canada. E-mail: saparicio@bccrc.ca

No conflicts of interest were declared.

Received: 1 September 2009
Revised: 23 September 2009
Accepted: 26 September 2009

Abstract

Next-generation DNA sequencing devices have revolutionized cancer genomics by bringing whole genome resequencing of patients' tumours within practical and economic reach. We present an overview of the techniques involved and review early results from the resequencing of cancer genomes. The possible impacts of whole-genome and trancriptome resequencing in clinical cancer research and the practice of pathology are discussed.

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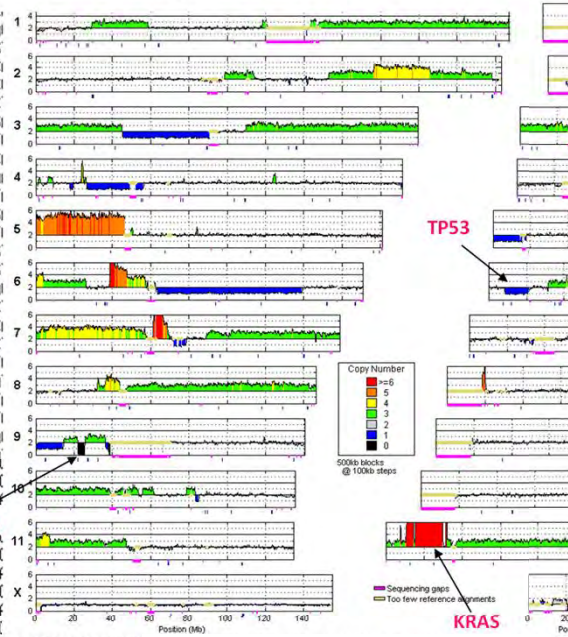
Keywords: DNA sequencing; mutational heterogeneity; cancer genome; transcriptome; tissue sampling; mutational landscape; tumour evolution

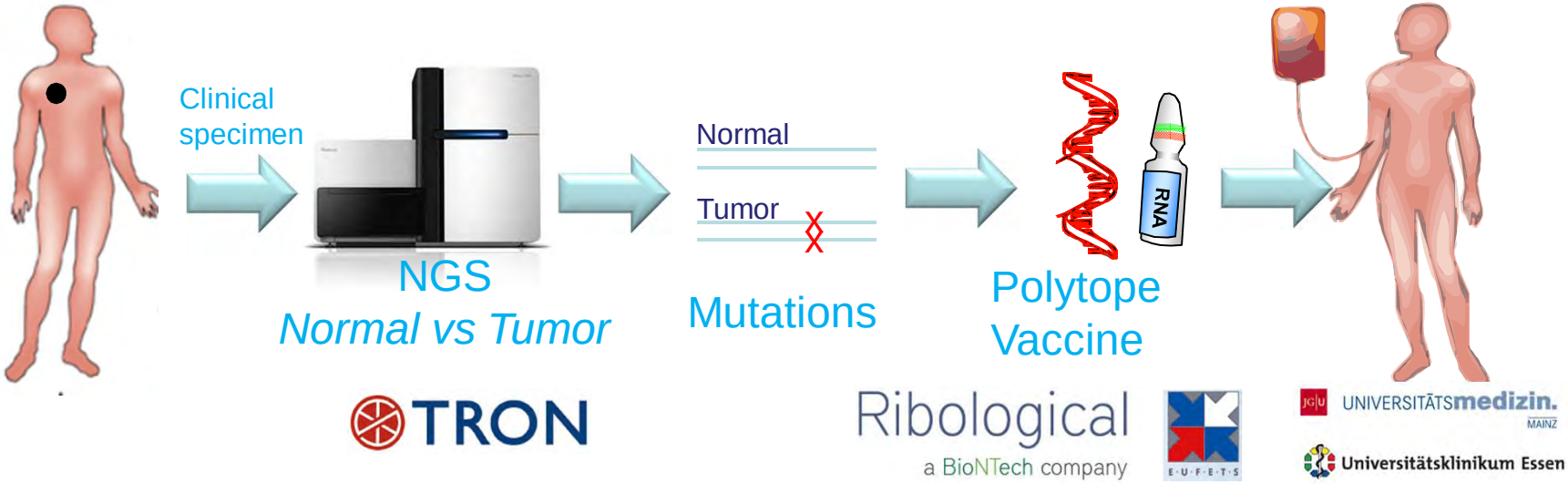
TRON's Illumina HiSeq2000 RNA & DNA sequencing



CNV in UMC-11 tumor cells

IVAC Individualisierte Vakzine

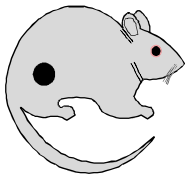
Von Mutationen zum individuell hergestellten Impfstoff



IVAC Präklinische Entwicklung

Von Mutationen zum individuell hergestellten Impfstoff

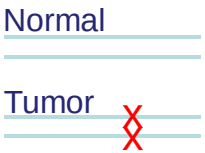
C57/B6 / B16 Melanoma
Balb/C / CT26 Colon Cancer



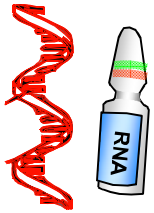
Tumor specimen

NGS
Normal vs Tumor

TRON

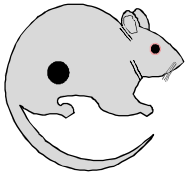


Mutations



Polytope Vaccine

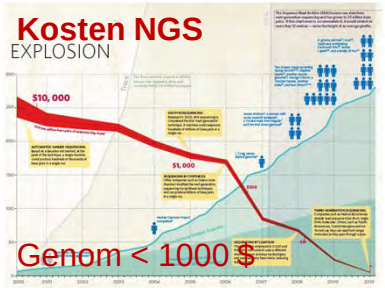
Ribological
a BioNTech company



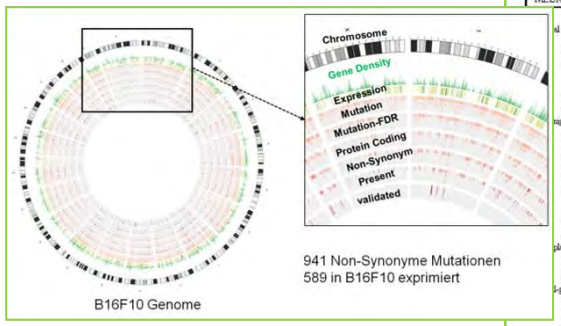
Immunogenicity
Tumor Inhibition
Feasibility / Safety

IVAC – Proof of Concept

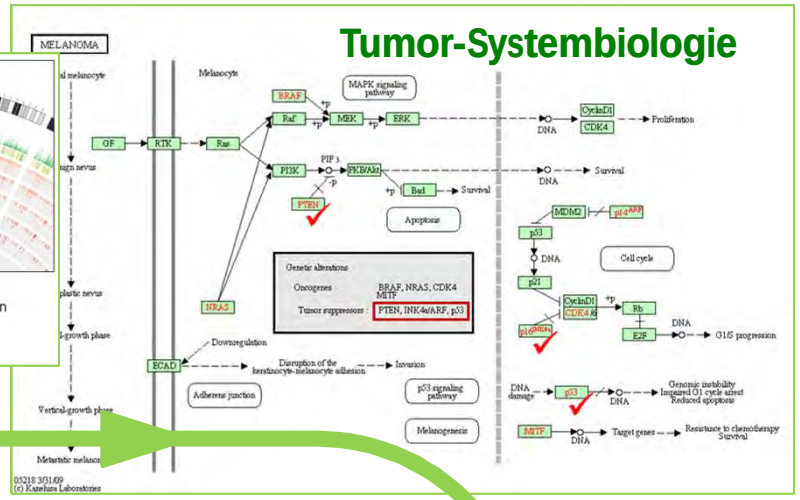
Next-Generation-Sequencing



Mutanom-Map



Tumor-Systembiologie

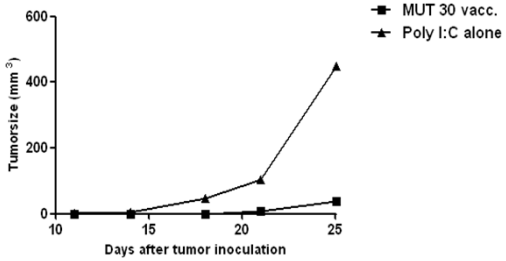


Herstelllung in wenigen Tagen ?

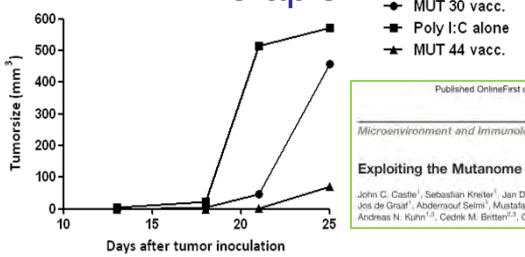
Mutation Specific Vakzine

Preclinical Efficacy

Prävention



Therapie



Published OnlineFirst on January 11, 2012; DOI:10.1158/0008-5472.CAN-11-3722

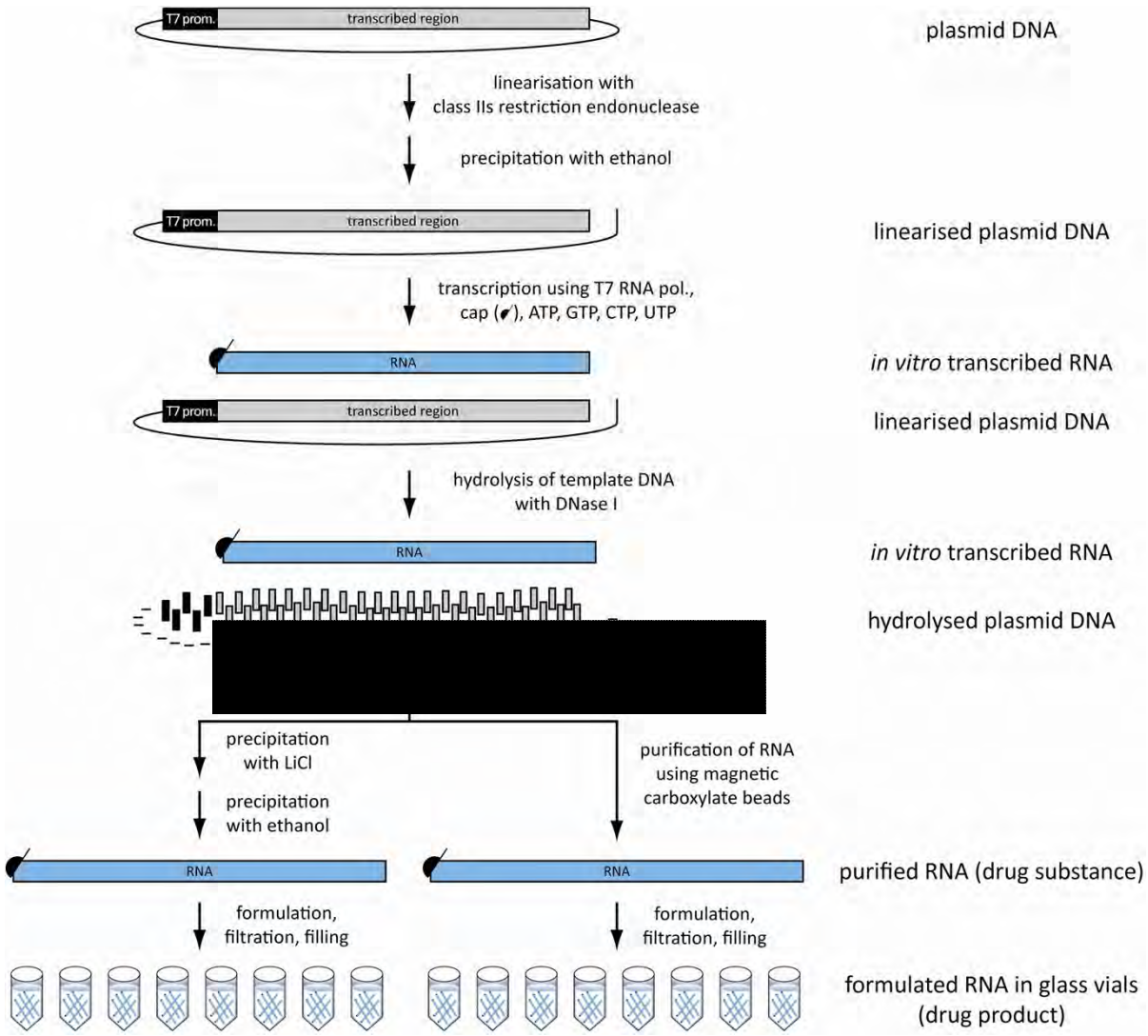
Microenvironment and Immunology

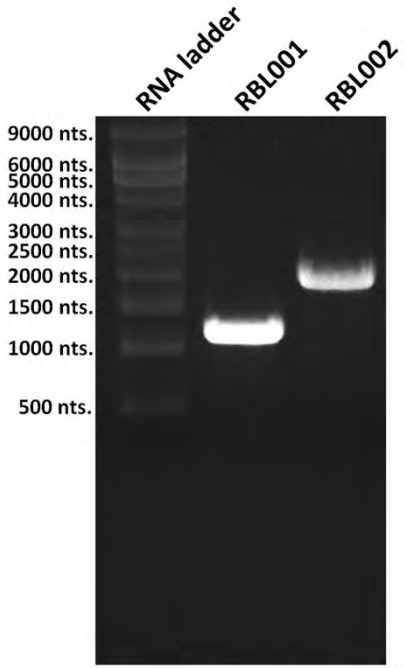
Cancer Research

Exploiting the Mutanome for Tumor Vaccination

John C. Castle, Sebastian Kreiter, Jan Diekmann, Martin Löwer, Niels van de Roemer, Jos de Graaf, Abderraouf Selmi, Mustafa Diken, Sebastian Boegel, Claudia Paret, Michael Koslowski, Andreas N. Kuhn, Cedrik M. Britten, Christoph Huber, Özlem Türeci, and Ugur Sahin

GMP Herstellung*





..Production Process can be done within days

* Cedrik Britten, Clinical Project Manager, A. Kuhn, Manufacturing Processes, GMP at EUFETS / Idar Oberstein

IVAC- Roadmap für klinische Translation

Bundesministerium für Bildung und Forschung

Innovative Molecular Therapies

C|3 Cluster for Individualized ImmunoIntervention

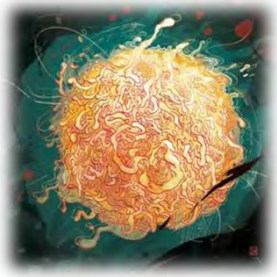
discovery → **preclinical development** → **First in Men 2013**

- **Center for Translational Oncology** TRON
- **Ribological** Ribological a BioNTech company BIONTECH
- **EUFETS** E·U·F·E·T·S
- **University Medical Centers** JG|U UNIVERSITÄTSmedizin. MAINZ; Universitätsklinikum Essen

Säulen Analytisch individualisierter Therapie

TRON

Patient Tumor



Target Discovery

Next Generation Sequenz.

Multiplex Analysen

Drug Development

Arzneimittel Warenhaus

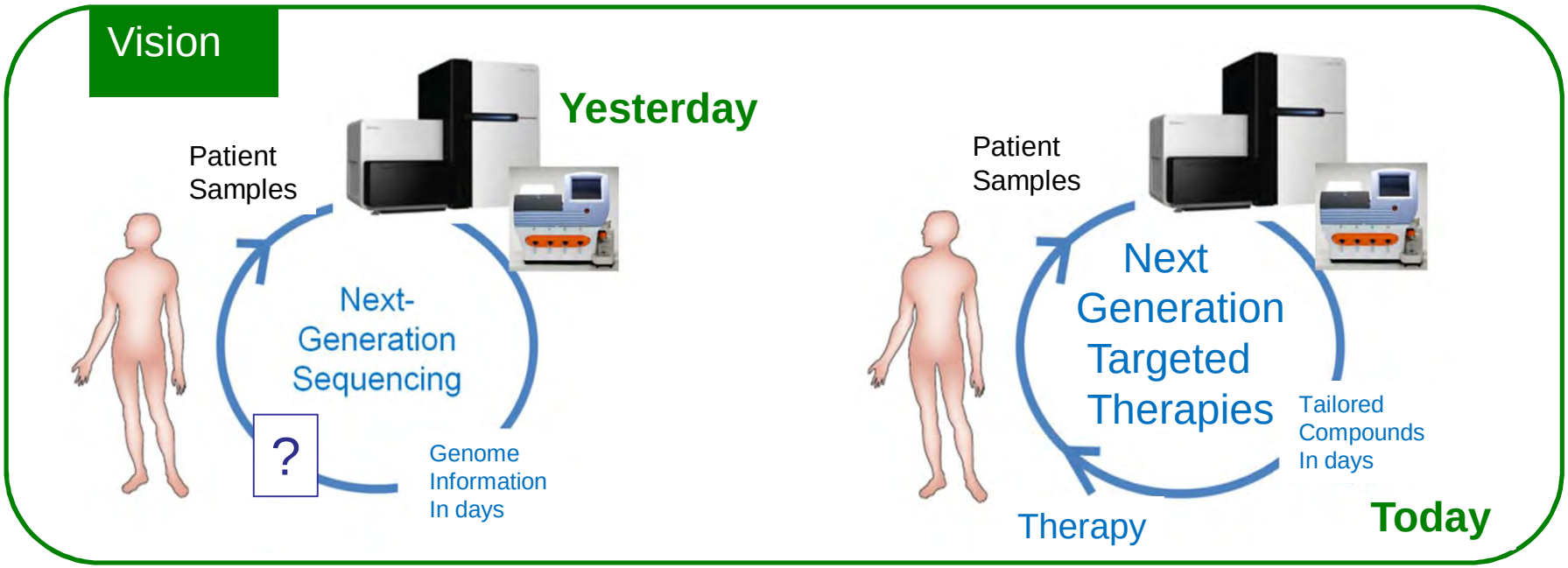
On Demand Herstellung

Kombination

Regulatory Environment

Patient Therapie

Zukünftige Therapie



TRON

Translation
Innovation
Think Tank

Immunology & RNA
Sebastian Kreiter
Mustafa Diken
Jan Diekmann
Niels van der Roemer
Sebastian Attig
Andreas Kuhn
Sebastian Petri
Janina Buck
Brian Davies

Sequencing & Validation
Valesca Boisguierin
Jos de Graaf
Claudia Paret

Computational Medicine
John Castle
Martin Löwer
Sebastian Boegel
Ludmilla Schemarov
Jelle Scholtebauers
Marius Byl

Development & Advice
Cedrik Britten, Björn Kloke, Birgit Pless, Sandra Heesch, Marino Schuhmacher, Alexander Kemmer-Brück
Christoph Huber, Özlem Türeci