肿瘤样本资源库在寻找生物标记物中的应用

Cancer Biobank: Biomarker Discovery

张 连 海

北京· 2013-12

Bridge the Gap between the Benchside Researchers and Bedside Clinicians

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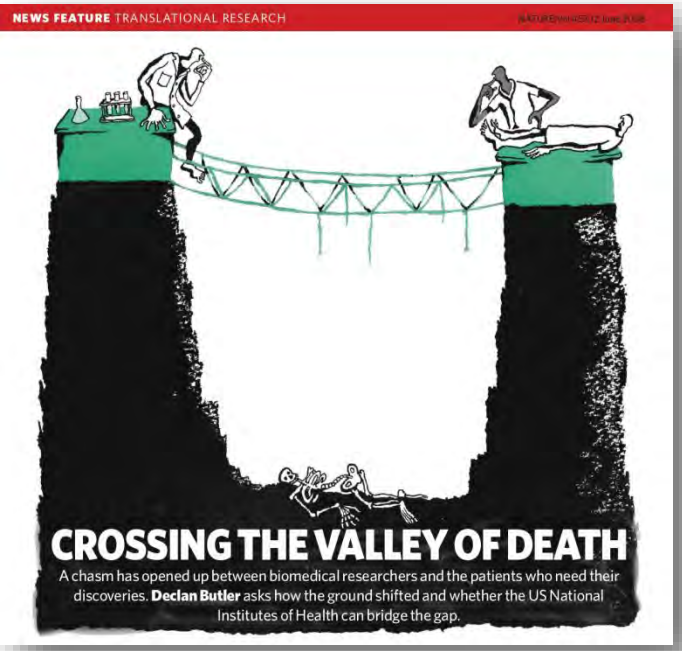

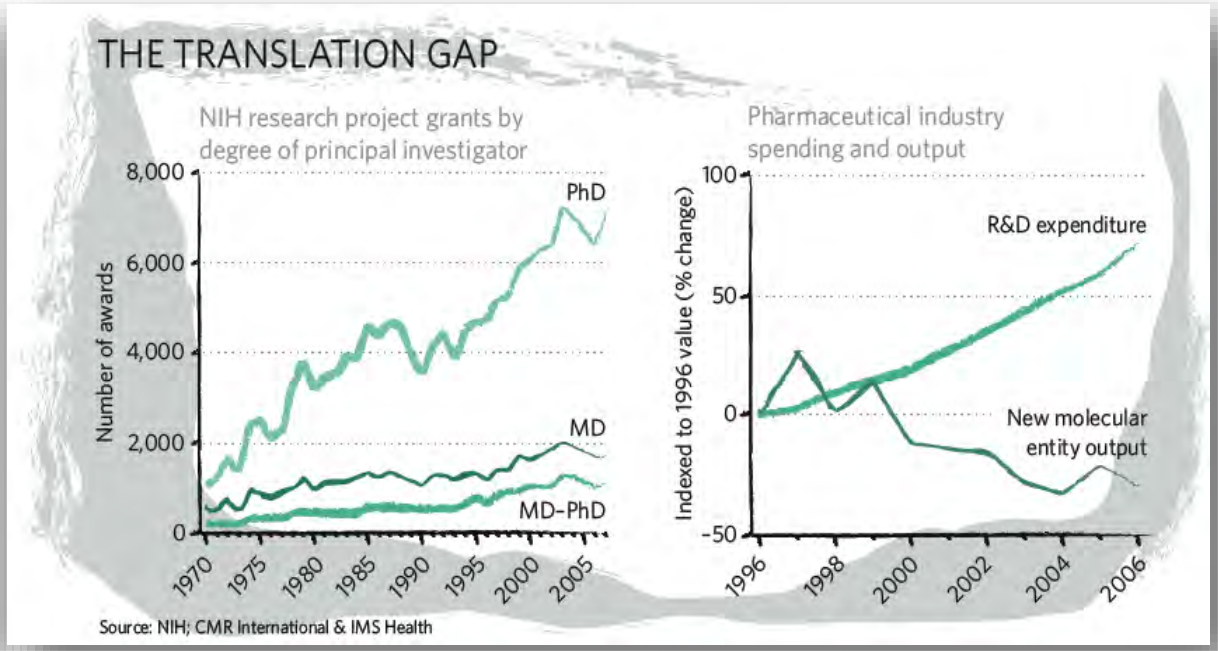

Butler D.
Translational research: crossing the valley of death.
Nature. 2008 Jun 12;453(7197):840-2.

Approaches

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Patients

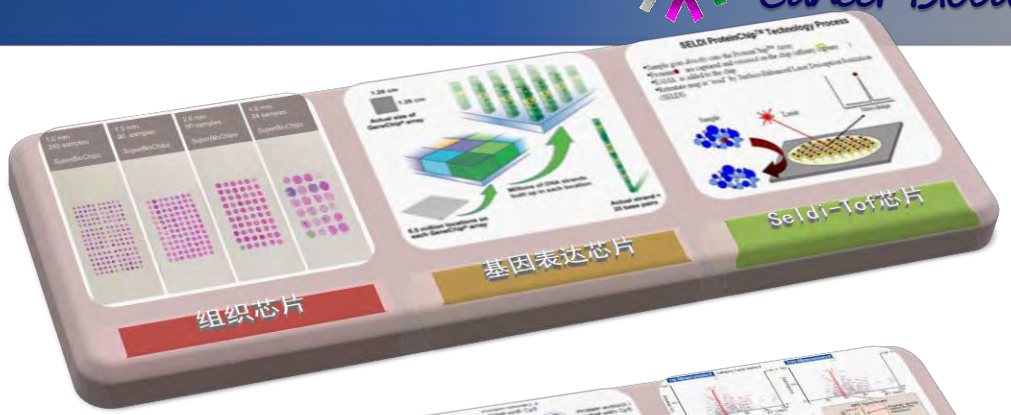
临床疗效评价
Clinical Evaluation

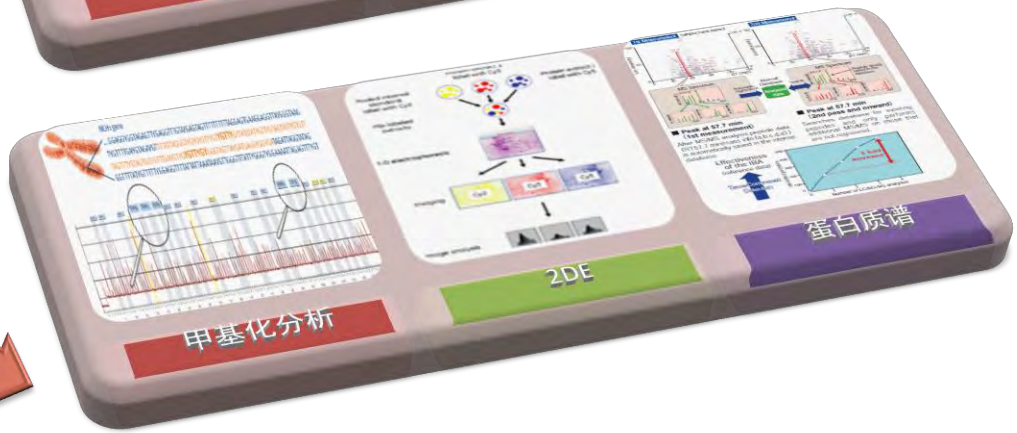
Standardized regimen

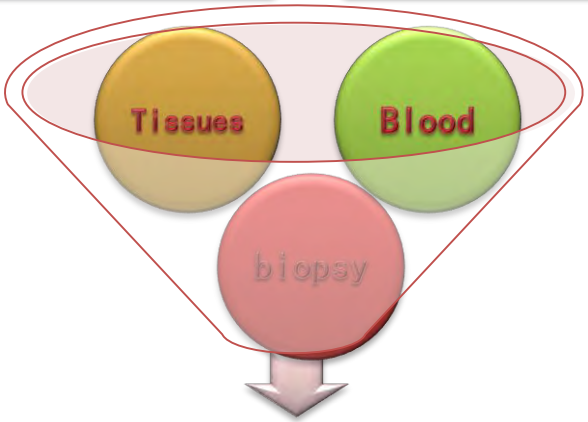
毒副作用评价
Side Effect Evaluation

Standardized surgery

病理学评价
Pathological Evaluation







Tissue bank

Response/Relapse prediction

Individualized treatment

From the Discovery to Recovery

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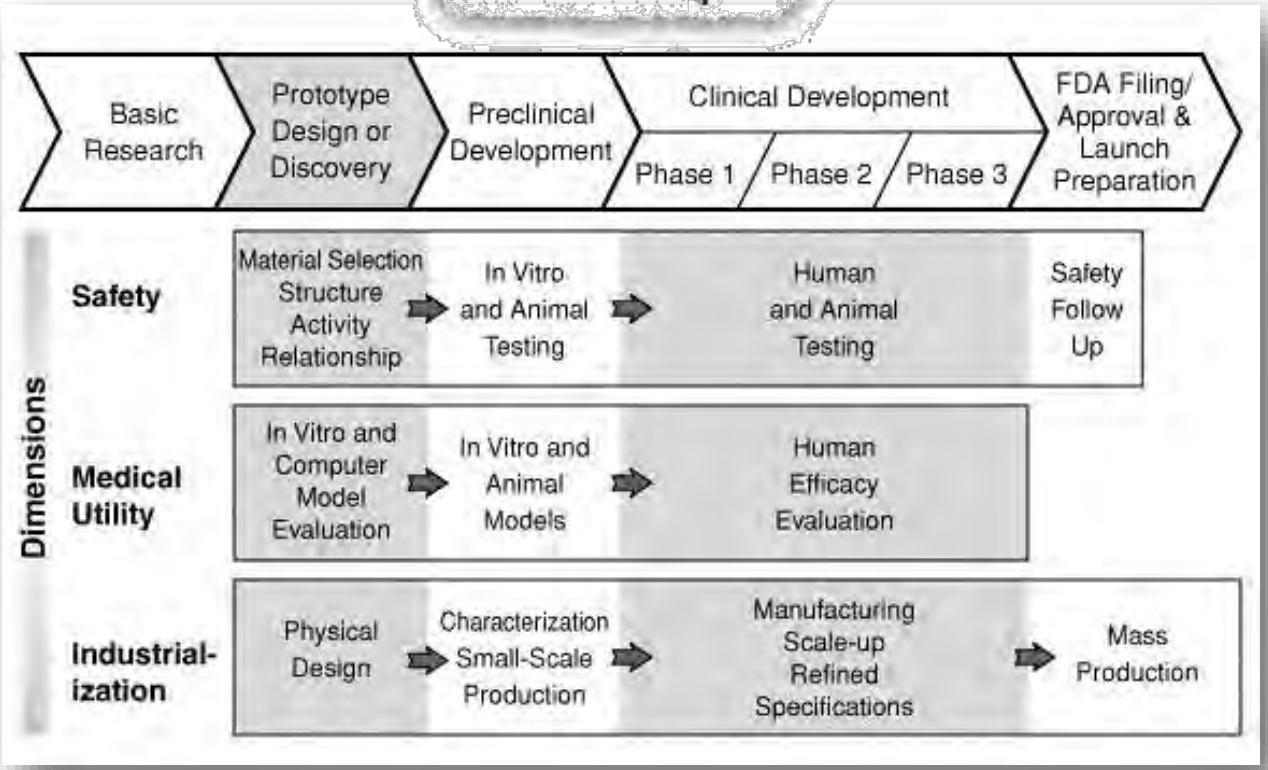

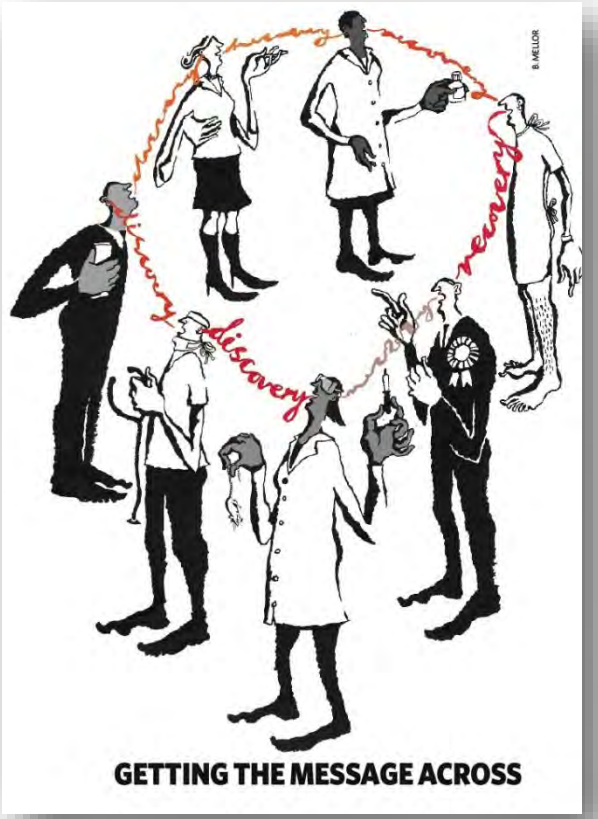

Anti-EGFR (Cetuximab) in Cancer

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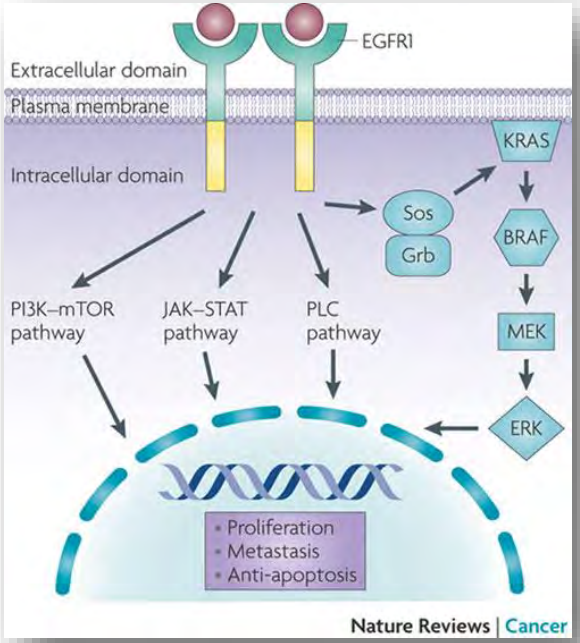
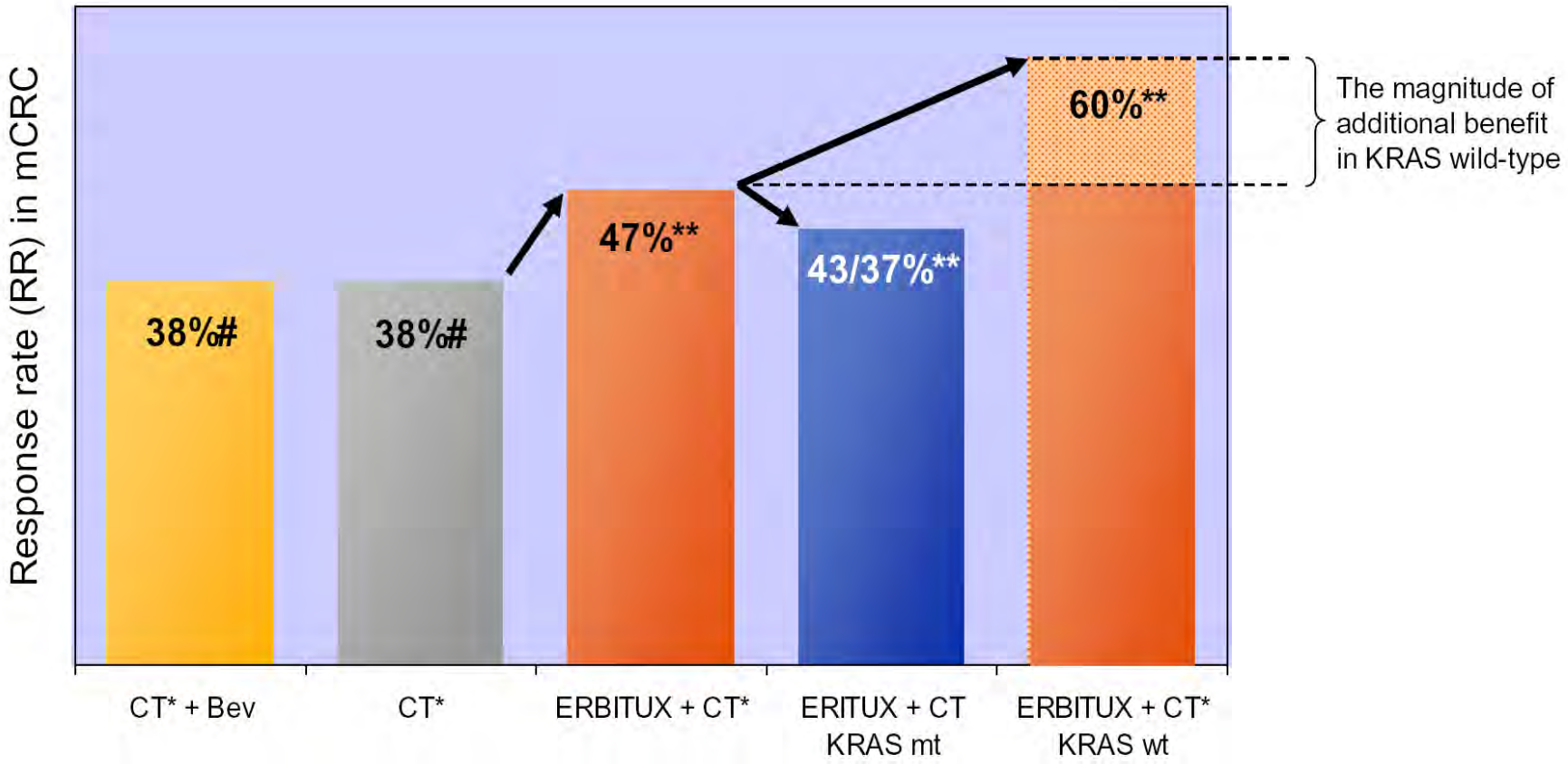


2004	**BOND** – Establishes 3rd-line therapy in mCRC; reversal of resistance
2005	**Bonner trial**: Introduces targeted therapy into LA H&N with RT
2006	**NCIC CO.17** – Only sign. survival ever demonstrated for a single agent in mCRC 3rd-line; only ever positive QoL data!
2006	The highest resection rates of liver mets ever reported >20% – Concept of increased resectability proposed
2007	**EXTREME**: First progress after 25 years, first targeted therapy introduced into metastatic H&N cancer
2007	**CRYSTAL**: Increased resectability first time demonstrated in phase III
2008	**CRYSTAL & OPUS**: KRAS will change treatment practice of mCRC
2008	**FLEX**: First progress of targeted therapy for all patients in 1st-line NSCLC

Impact of KRAS on Activity (RR) of 1st-line mCRC Treatments for Illustration



*Standard CT: FOLFOX/FOLFIRI **data from CRYSTAL/OPUS #NO16966

结果：两组K-RAS突变情况

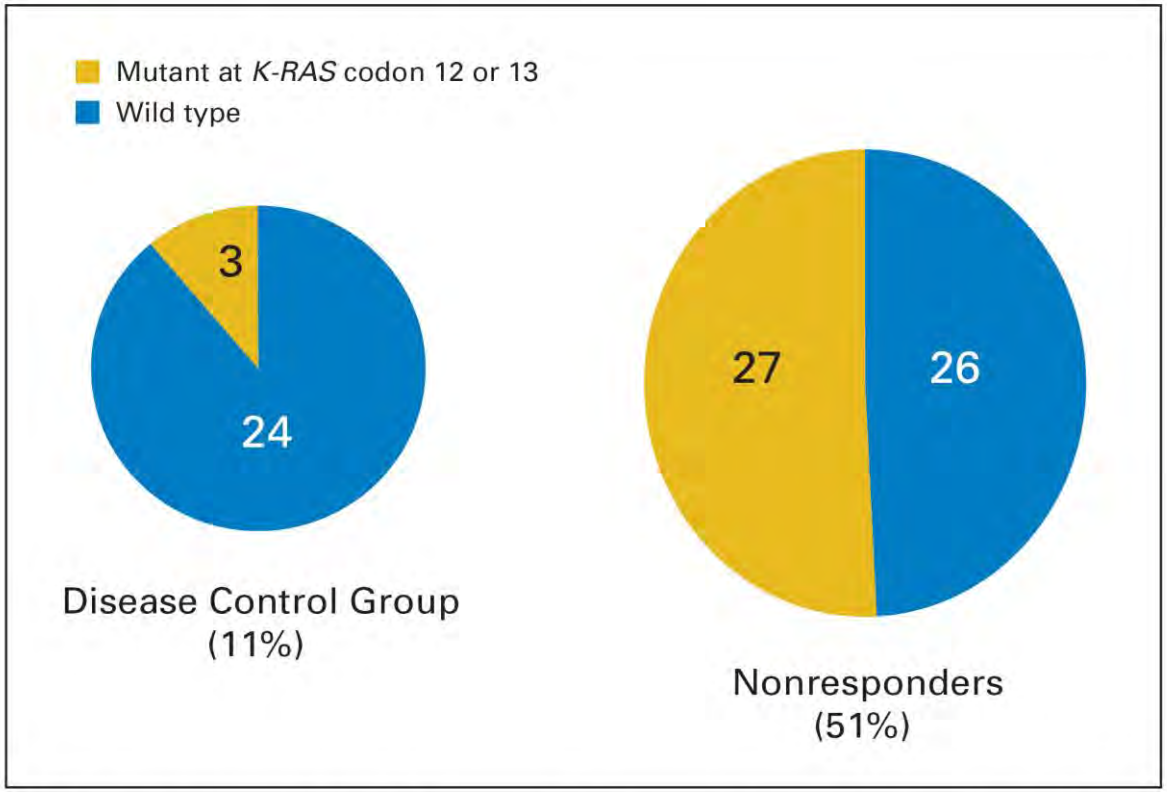


Fig 4. Mutation analysis of *K-ras* exon 2. *K-ras* mutations were detected in three patients with stable disease (SD) out of 27 disease control group patients (five partial responses + 22 SD). *K-ras* mutations were detected in 27 of 53 nonresponders. There is a statistically significant difference in *K-ras* mutation frequency between the two groups (Fisher's exact test, $P = .0003$).

Khambata-Ford S, et al. J Clin Onc 2007;25:3230–7

回顾性研究支持此观点：在化疗无效的转移性结直肠癌病人中，KRAS突变与对EGFR阻断剂无反应之间存在一定关联

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Reference	Treatment	No. of patients (wild-type:mutant)	Objective response n (%)	
			Wild-type	mutant
A Lièvre *et al*, *(J Clin Oncol 2008)*	Cetuximab ± CT	114 (78:36)	34 (44)	0 (0)
S Benvenuti *et al*, *(Cancer Res 2007)*	Panitumumab or cetuximab or Cetuximab + CT	48 (32:16)	10 (31)	1 (6)
W DeRoock, E Van Cutsem S Tejpar *(Ann Oncol 2008)*	Cetuximab or Cetuximab + irinotecan	113 (67:46)	27 (41)	0 (0)
D Finocchiaro *et al*, *(ASCO Proceedings 2007)*	Cetuximab ± CT	81 (49:32)	13 (26)	2 (6)
F Di Fiore *et al*, *(Br J Cancer 2007)*	Cetuximab + CT	59 (43:16)	12 (28)	0 (0)
S Khambata-Ford *et al*, *(J Clin Oncol 2007)*	Cetuximab	80 (50:30)	5 (10)	0 (0)
RG Amado *et al*, *(J Clin Oncol 2008)*	Panitumumab	208 (124:84)	21 (17)	0 (0)

The Importance of KRAS and BRAF Testing in Colorectal Cancer…and more PTEN/PIK3CA

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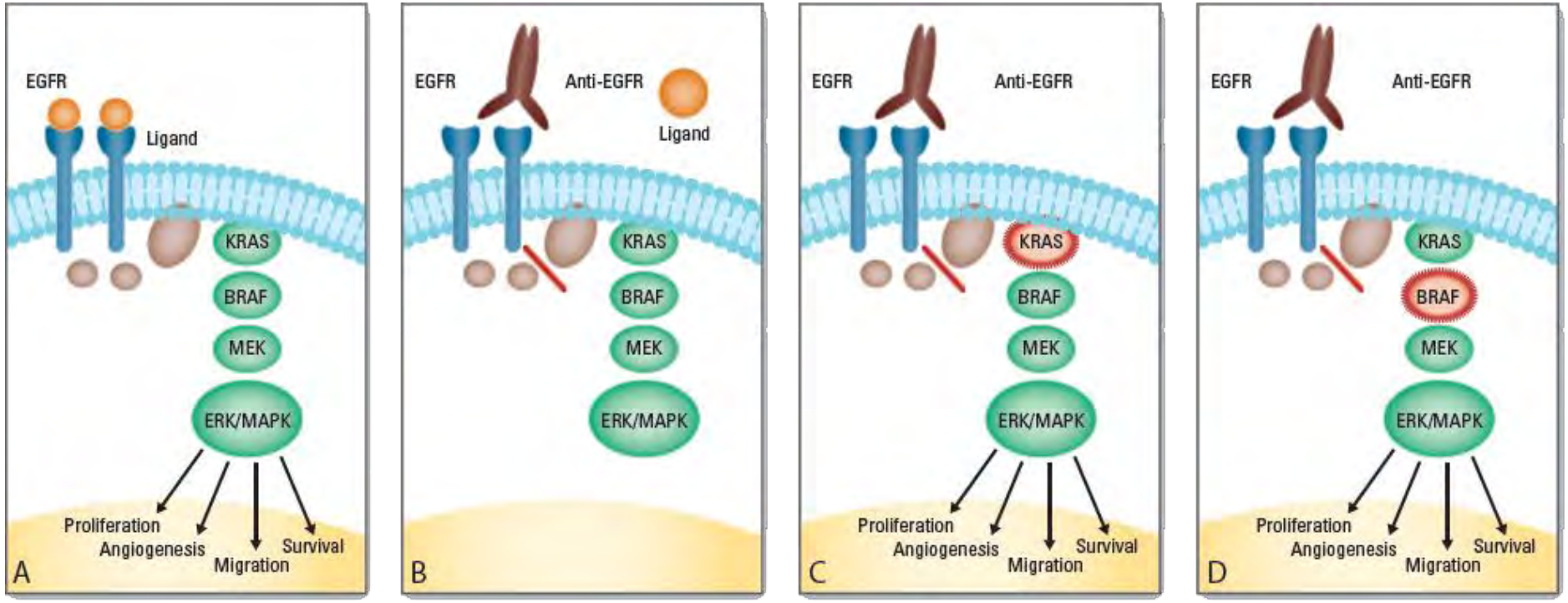

Graphic representation of a cohort of 100 patients with colorectal cancer treated with cetuximab

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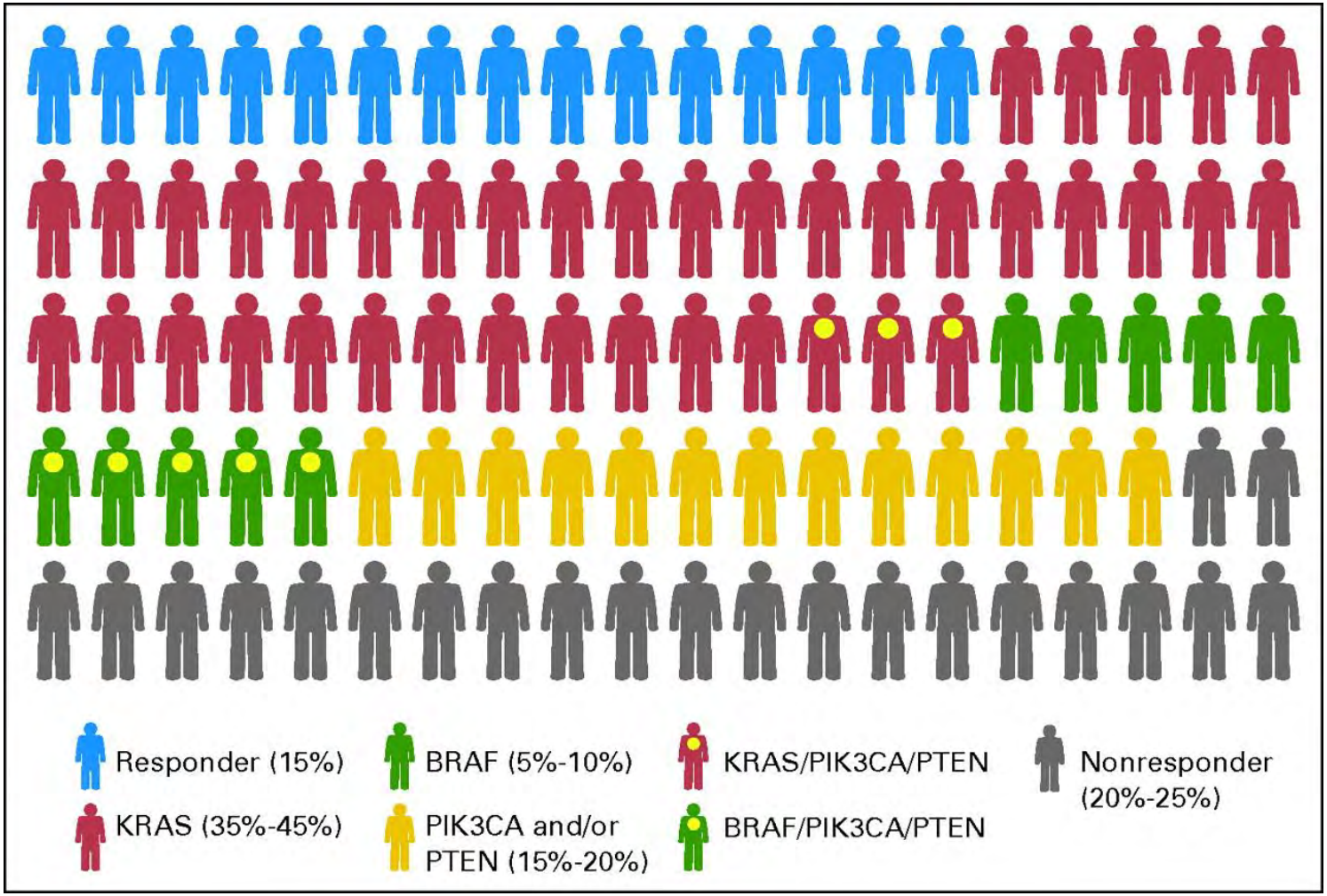
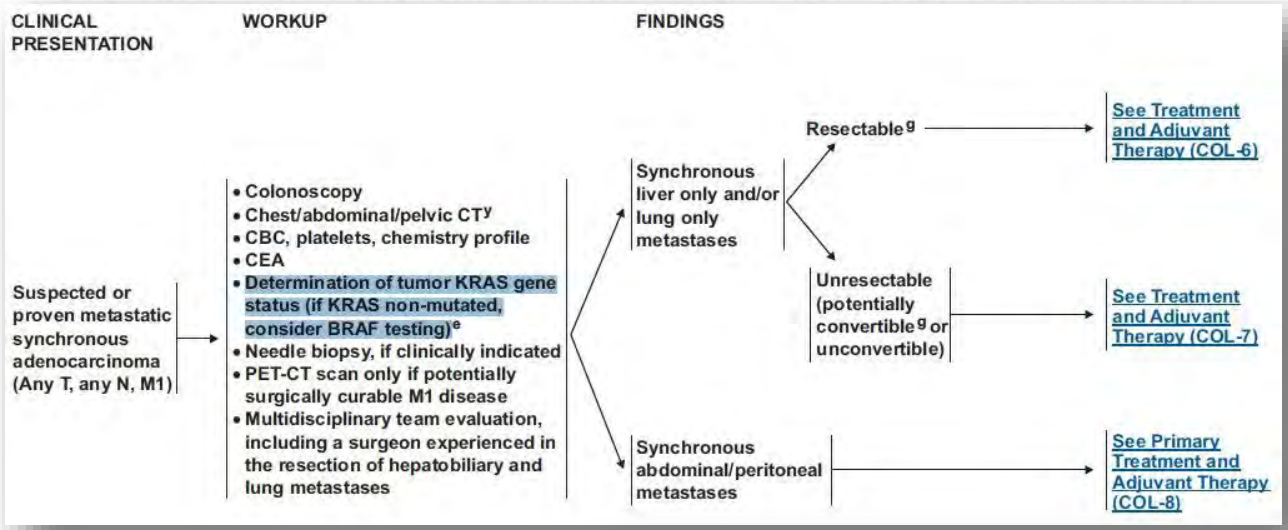


Bardelli A , Siena S JCO 2010;28:1254-1261

JOURNAL OF CLINICAL ONCOLOGY

BRAF Mutation Testing

- **Patients with a V600E BRAF mutation appear to have a poorer prognosis. Retrospective subset analyses suggest potential benefit from anti-EGFR monoclonal antibodies in the first-line setting with active chemotherapy regardless of V600E mutation status. Limited available data suggest lack of antitumor activity from anti-EGFR monoclonal antibodies in the presence of a V600E mutation when used after patient has progressed on first-line therapy.[46,47]**
- **Testing for the BRAF V600E mutation can be performed on formalin fixed paraffin embedded tissues. This is usually performed by PCR amplification and direct DNA sequence analysis. Allele-specific PCR is another acceptable method for detecting BRAF V600E mutation. This testing should be performed only in laboratories that are certified under the clinical laboratory improvement amendments of 1988 (CLIA-88) and qualified to perform highly complex clinical laboratory (molecular pathology) testing.**



[g] See Principles of Surgery (COL-B 2 of 3).
[ee] Determination of tumor KRAS (if KRAS non-mutated, consider BRAF testing). See Principles of Pathologic Review (COL-A 4 of 5) - KRAS and BRAF Mutation Testing.

在使用抗EGFR 单克隆抗体进行治疗之前，先取原发肿瘤组织或转移肿瘤组织样本进行基因突变检测，对KRAS 野生型的患者增加BRAF 突变的检测

对于存在BRAF V600E 突变的患者，不推荐用抗EGFR 单克隆抗体进行治疗

KRAS突变检测的报告以及标准流程（SOP）

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KRAS 第二外显子（第 12、13 编码子）突变检测的报告以及标准流程（SOP）

患者姓名： ZKW 诊断： 直肠癌 检测标本： 石蜡切片 时间： 2008-11-10 检测人： ZLH

EGFR 免疫组化结果 未知

检测流程：

阴性对照（可选）： 外周血（抗凝）→ 提取 DNA （Qiagen 69504）→ 浓度测定 60 ng/ul → PCR 反应 x 1

阳性对照： 目前已经有经测序证实的突变样本，每次测试必须加阳性对照样本 → PCR 反应 x 1；

待测样本： 穿刺活检组织/石蜡切片→ 提取 DNA （Qiagen 69504）→ 浓度测定 20 ng/ul → PCR 反应 x 2

**由于肿瘤组织中含有一定量的正常组织（wtKRAS），因此活检尽量选择肿瘤含量丰富的区域进行；石蜡切片尽量选取一张作 HE 染色，以判定肿瘤的大致范围，然后在其他切片上尽量刮取肿瘤细胞多的区域。*

PCR 反应条件 (50μl)

Component	Final Volume	Final Concentration
5X Green GoTaq® Flexi Buffer (M891A)	10μl	1X
$MgCl_2$ Solution, 25mM (A351B)	3μl	1.5mM
PCR Nucleotide Mix, 2.5mM each	4μl	0.2mM each dNTP
upstream primer 100μM	0.5μl	1.0μM
downstream primer 100μM	0.5μl	1.0μM
GoTaq® DNA Polymerase (5u/μl) (M829A)	0.25μl	1.25u
template DNA	Xμl	<500ng/50μl
Nuclease-Free Water	to 50μl	

Step	Temperature	Time	Number of Cycles
Initial Denaturation	95°C	2 minutes	1 cycle
Denaturation	95°C	30"	
Annealing	60°C	30"	**35 cycles**
Extension	72°C	20"	
Final Extension	72°C	5 minutes	1 cycle

KRAS突变检测的报告

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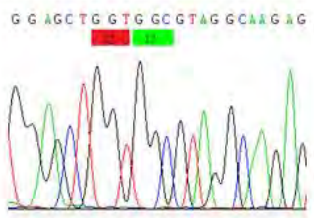
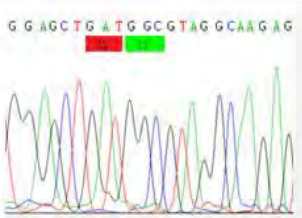
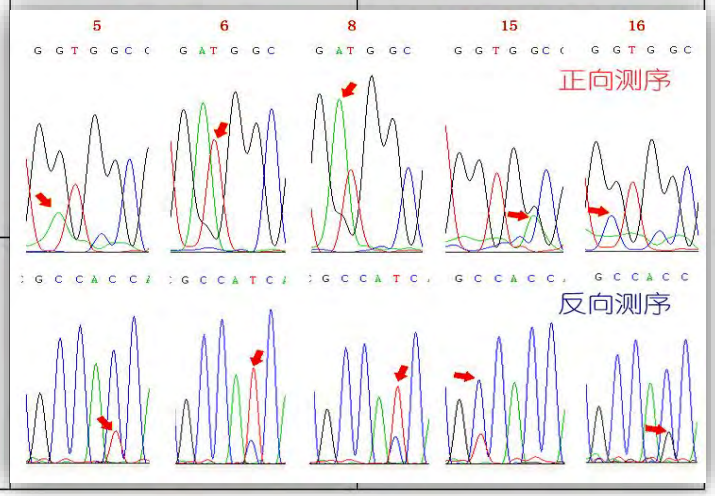
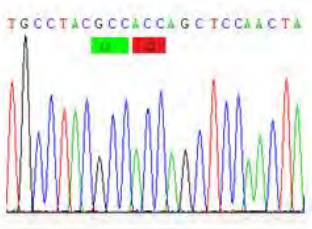
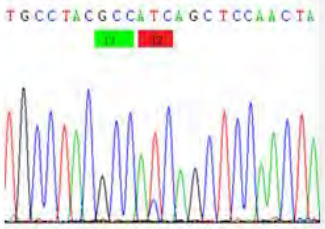
反应产物测序 （正向+反向）结果：

没有/有突变。

	阴性对照（外周血）	阳性对照（样本 CR6）	活检组织 1	活检组织 2
正向				
反向				

参考序列来源：

Homo sapiens v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) gene, complete cds 49663 bp DNA linear PRI 06-FEB-2008

http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&id=166706780

引物设计：

TCTAATATAG TCACATTTTC ATTATTTTTA TTATAAGGCC TGCTGAAAAT GACTGAATAT AAACTTGTGG TAGTTGGAGC TGGTGGCGTA GGCAAGAGTG CCTTGACGAT

ACAGCTAATT CAGAATCATT TTGTGGACGA ATATGATCCA ACAATAGAGG TAAATCTTGT TTTAATATGC ATATTACTGG TGCAGGACCA TTCTTTGATA CAGATAAAGG

注：阴影区域为第二外显子；蓝色字体为引物序列；GGT第十二编码子；GGC第十三编码子

引物序列：5’-GGCCT GCTGA AAATG ACTGA-3’；5’-GTCCT GCACC AGTAA TATGC-3’

Anti-HER2 (Herceptin) in Gastric Cancer

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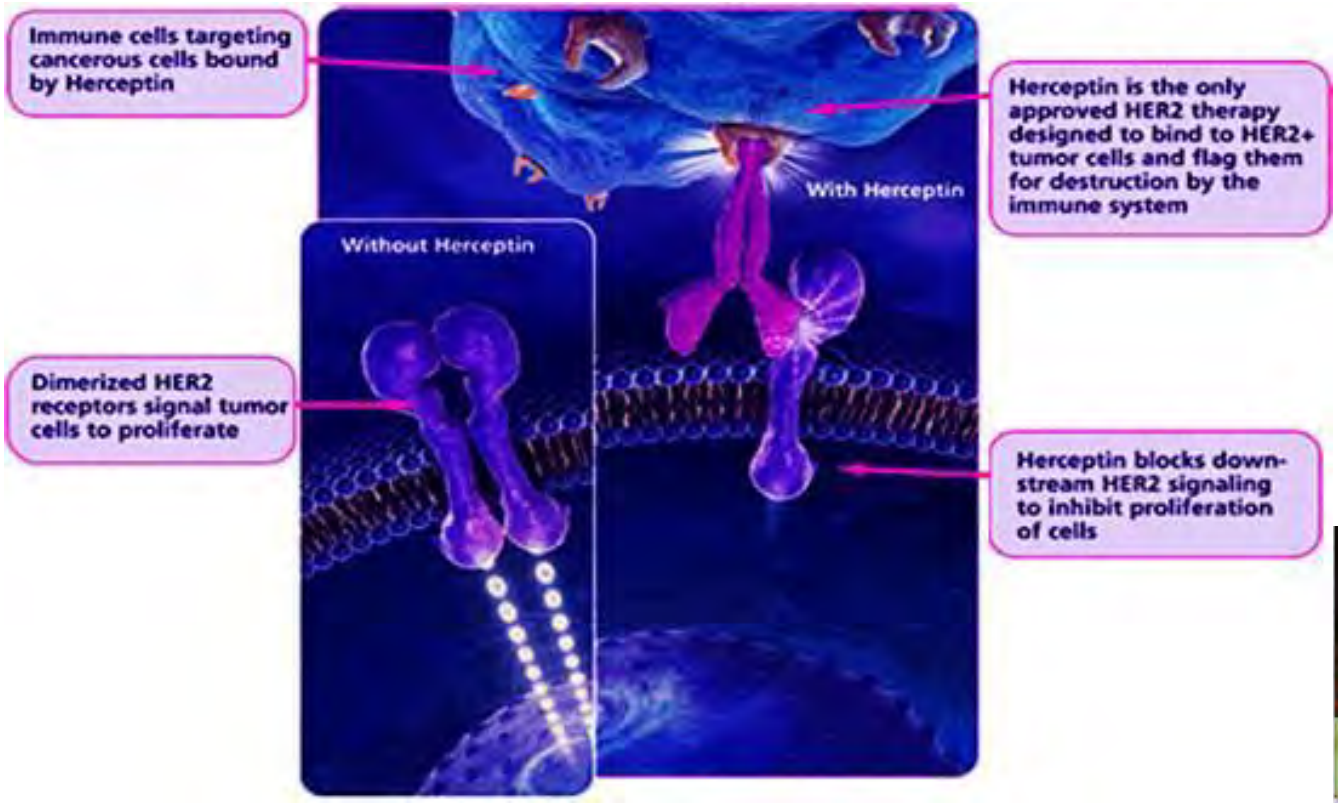

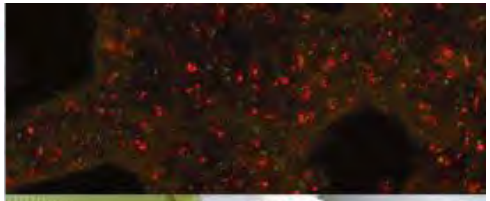

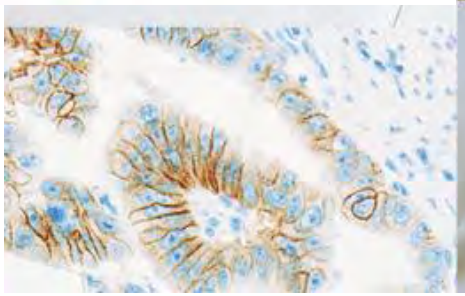

ToGA Trial

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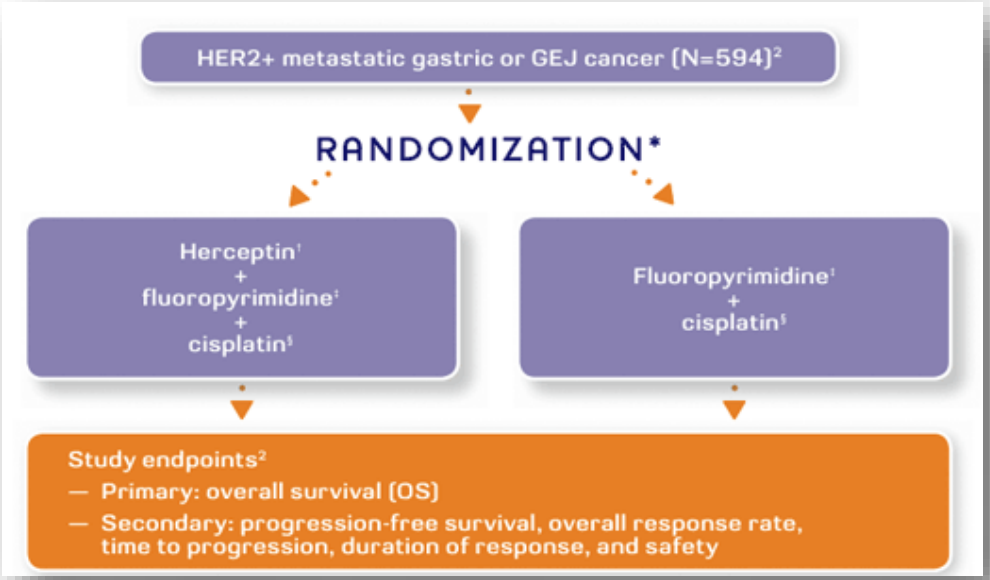
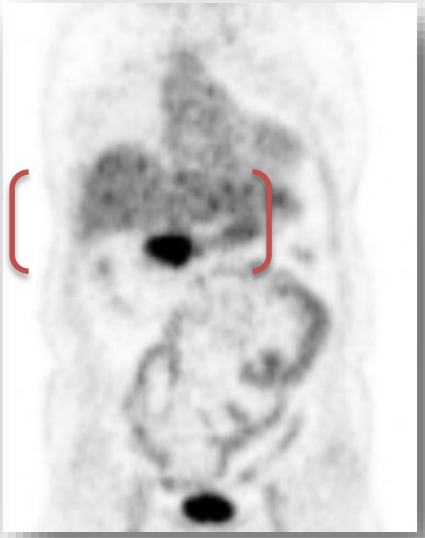
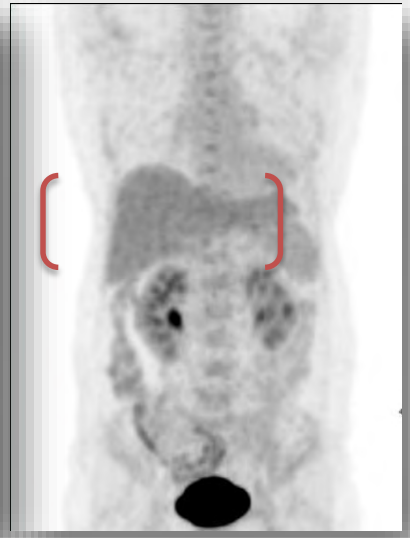




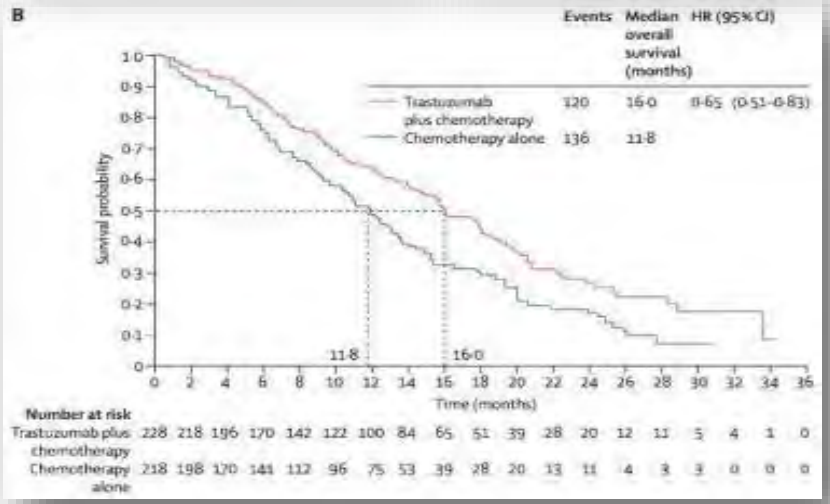


THE LANCET

The Lancet, Volume 376, Issue 9742, Pages 687 - 697, 28 August 2010

Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial

Prof Yung-Jue Bang MD [a] *, Prof Eric Van Cutsem MD [b] *, Andrea Feyereislova MD [c], Prof Hyun C Chung MD [d], Prof Lin Shen MD [e], Akira Sawaki MD [f], Florian Lordick MD [g], Atsushi Ohtsu MD [h], Yasushi Omuro MD [i], Taroh Satoh MD [j], Giuseppe Aprile MD [k], Evgeny Kulikov MD [l], Julie Hill PhD [m], Michaela Lehle PhD [c], Prof Josef Rüschoff MD [n], Prof Yoon-Koo Kang MD [o], for the ToGA Trial Investigators[†]



Roche/Genentech Global Gastric Cancer Clinical Advisory Board Meeting

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Advisors		
Carlo Barone	Atsushi Ohtsu	Yung-Jue Bang
Eric van Cutsem	Joseph Rüschoff	
Jia-Fu Ji	Manish Shah	David Cunningham
Florian Lordick	Josep Taberner	
Atsushi Ochiai	Marc Ychou	Yoon-Koo Kang

① GC treatment based on HER2 status

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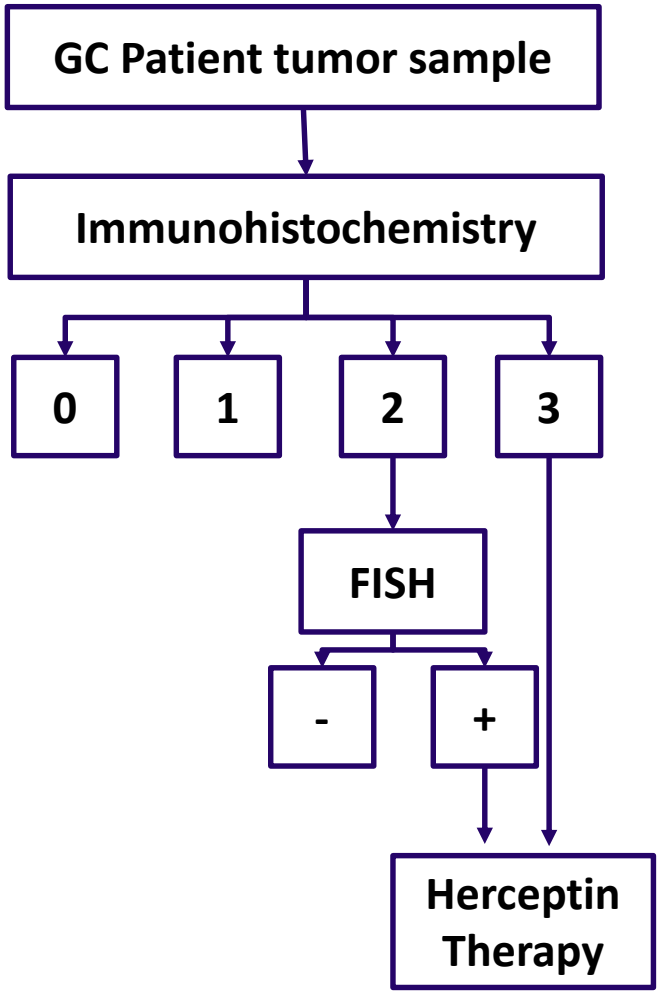

Preliminary Results

Sample Analysis→ Surrogate Biomarker

Clinical Trial

Clinical Guideline

NCCN Guide for Metastatic and Locally Advanced Gastric Cancer

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Metastatic or Locally Advanced Cancer

(where chemoradiation is not recommended):

- DCF (Docetaxel, cisplatin and 5-FU) (category 1)[6]
- ECF (category 1)[7]
- ECF modifications (category 1)[2,8,9]
- Irinotecan plus cisplatin (category 2B)[10,11]
- Oxaliplatin plus fluoropyrimidine (5-FU† or capecitabine) (category 2B)[8,12]
- DCF modifications (category 2B)[2,13,14,15]
- Irinotecan plus fluoropyrimidine (5-FU or capecitabine) (category 2B)[16,17]
- Paclitaxel-based regimen (category 2B)
- Trastuzumab††,[18]

NCCN National Comprehensive Cancer Network®

The Importance of Individualized Medicine

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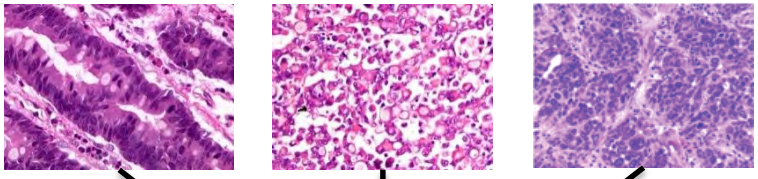
Now

case1 case2 case3

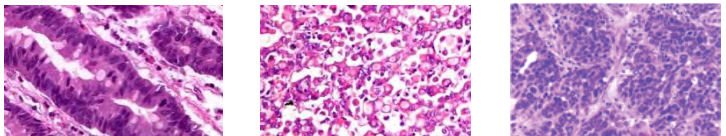


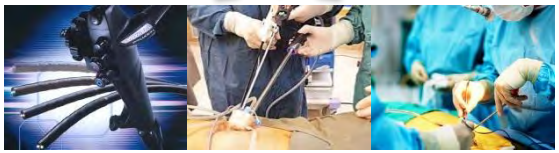
Standardized treatment

Only partial benefit

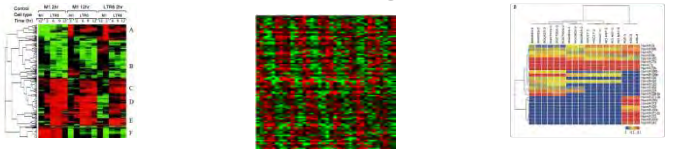
Future

case1 case2 case3





Molecular Diagnosis



Treatment a

Treatment b

Treatment c

Preclinical Investigation on Patient Derived Xenograft (PDX) Animal Models of GC

EGFR Targeting Drug in Gastric Cancer: Negative

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Lancet Oncol. 2013 May;14(6):490-9. doi: 10.1016/S1470-2045(13)70102-5. Epub 2013 Apr 15.

Capecitabine and cisplatin with or without cetuximab for patients with previously untreated advanced gastric cancer (EXPAND): a randomised, open-label phase 3 trial.

Lordick F, Kang YK, Chung HC, Salman P, Oh SC, Bodoky G, Kurteva G, Volovat C, Moiseyenko VM, Gorbunova V, Park JO, Sawaki A, Celik I, Götte H, Melezínková H, Moehler M; Arbeitsgemeinschaft Internistische Onkologie and EXPAND Investigators.

Collaborators (153)

FINDINGS: Between June 30, 2008, and Dec 15, 2010, we enrolled 904 patients. Median PFS for 455 patients allocated capecitabine-cisplatin plus cetuximab was 4.4 months (95% CI 4.2-5.5) compared with 5.6 months (5.1-5.7) for 449 patients who were allocated to receive capecitabine-cisplatin alone (hazard ratio 1.09, 95% CI 0.92-1.29; p=0.32). 369 (83%) of 446 patients in the chemotherapy plus cetuximab group and 337 (77%) of 436 patients in the chemotherapy group had grade 3-4 adverse events, including grade 3-4 diarrhoea, hypokalaemia, hypomagnesaemia, rash, and hand-foot syndrome. Grade 3-4 neutropenia was more common in controls than in patients who received cetuximab. Incidence of grade 3-4 skin reactions and acne-like rash was substantially higher in the cetuximab-containing regimen than in the control regimen. 239 (54%) of 446 in the cetuximab group and 194 (44%) of 436 in the control group had any grade of serious adverse event.

INTERPRETATION: Addition of cetuximab to capecitabine-cisplatin provided no additional benefit to chemotherapy alone in the first-line treatment of advanced gastric cancer in our trial.

THE LANCET Oncology
Volume 14, Issue 6, May 2013, Pages 490–499

2- Preclinical Trial
Cetuximab in Gastric Cancer

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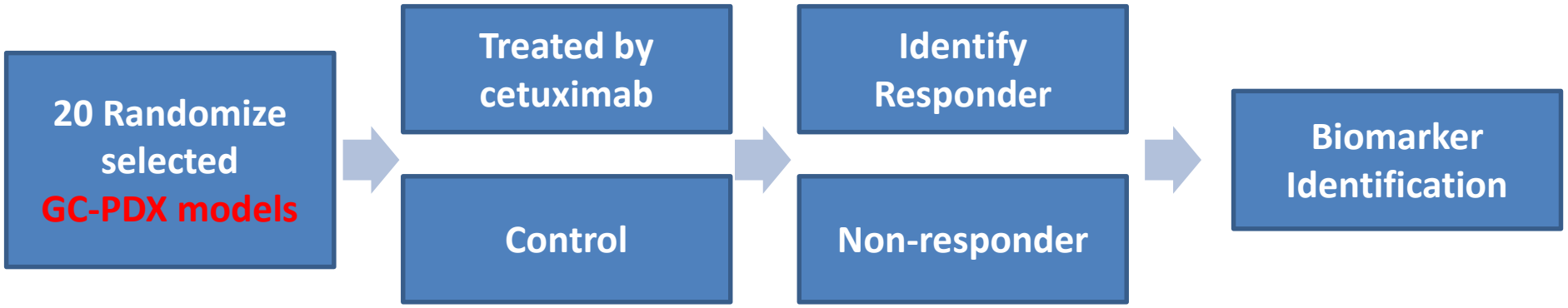
Cancer Biobank



Responders in animal models

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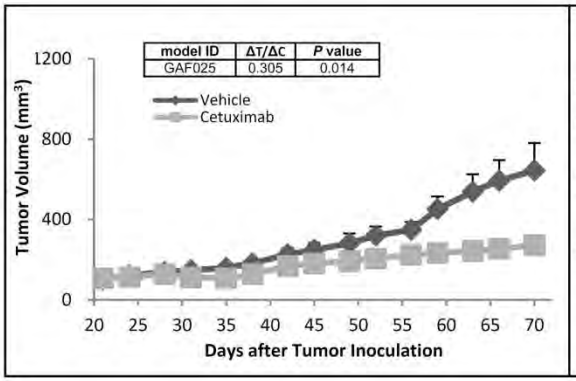

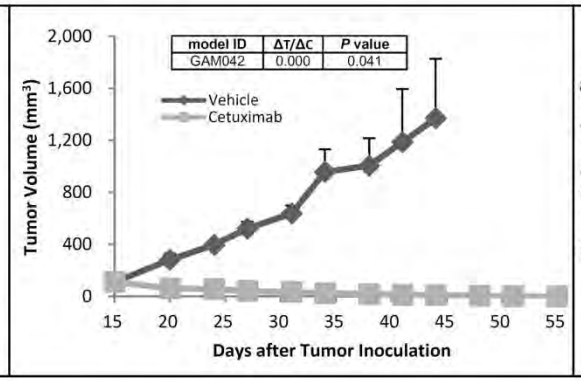

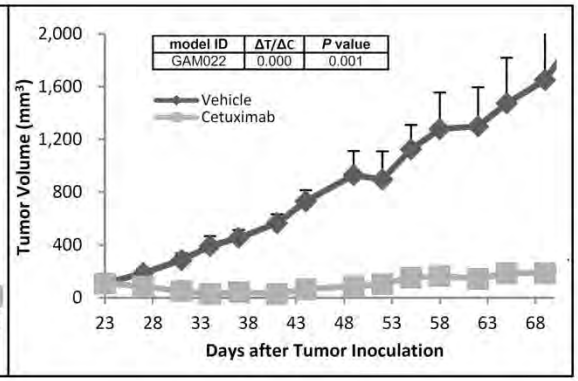

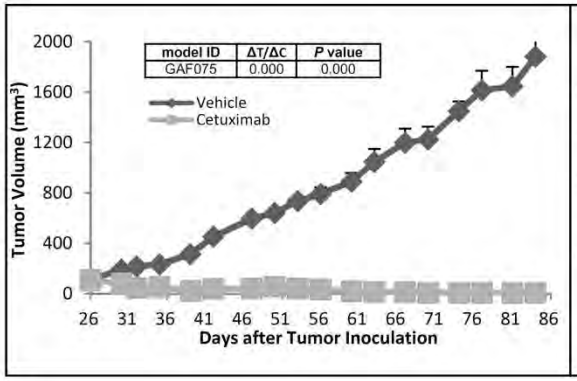

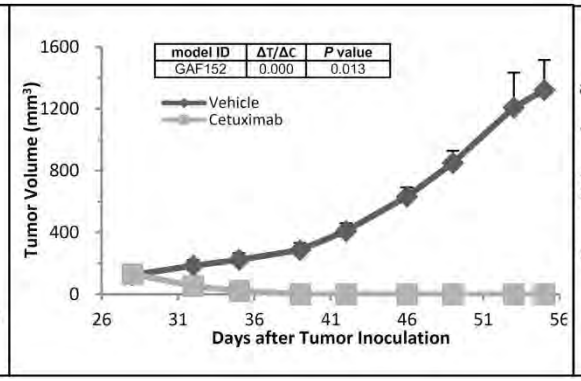

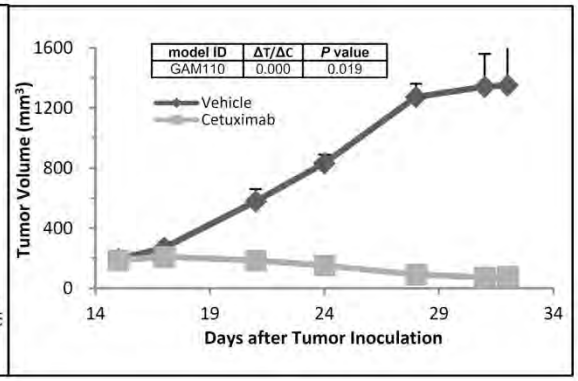

No responder

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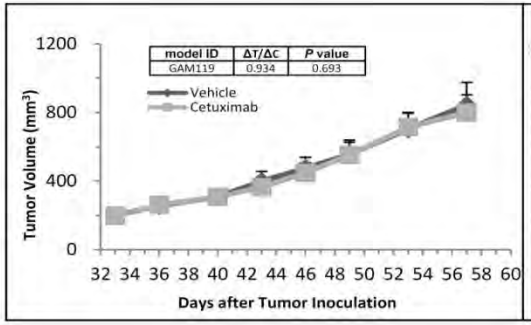
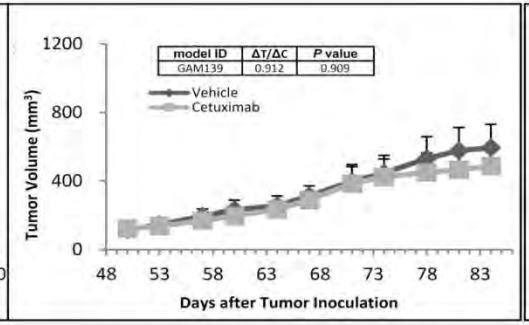
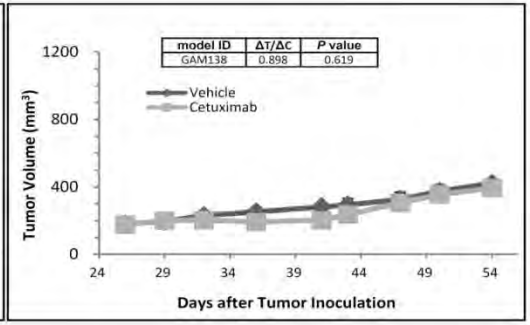
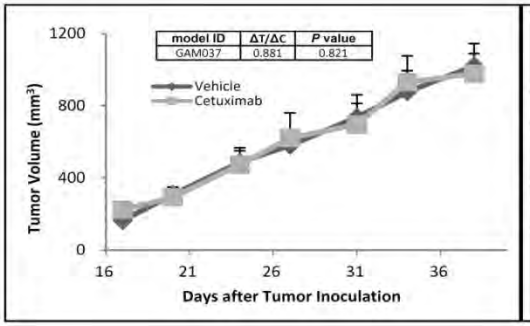
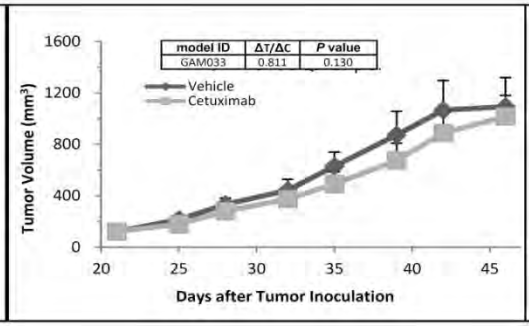
Cancer Biobank

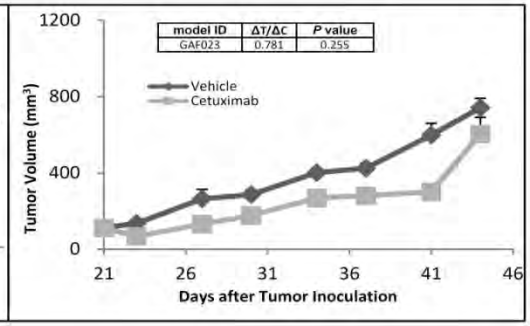


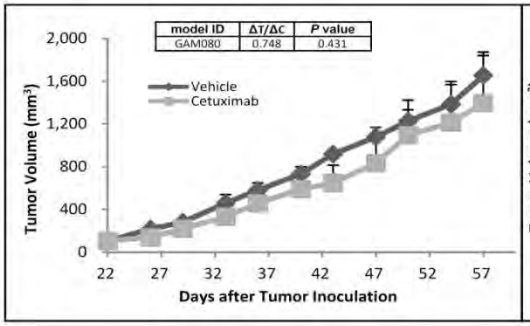


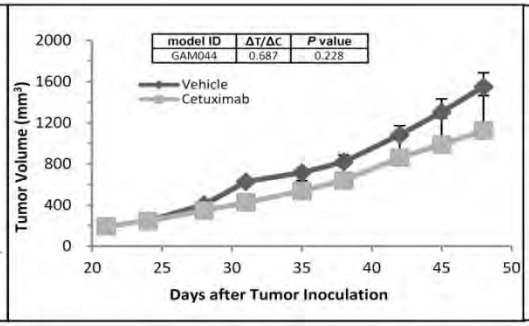


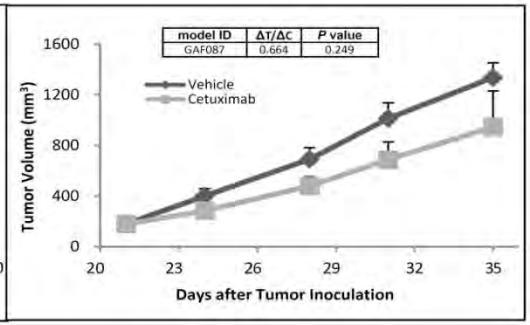












The anti-EGFR treatment affects EGFR expression (IHC)

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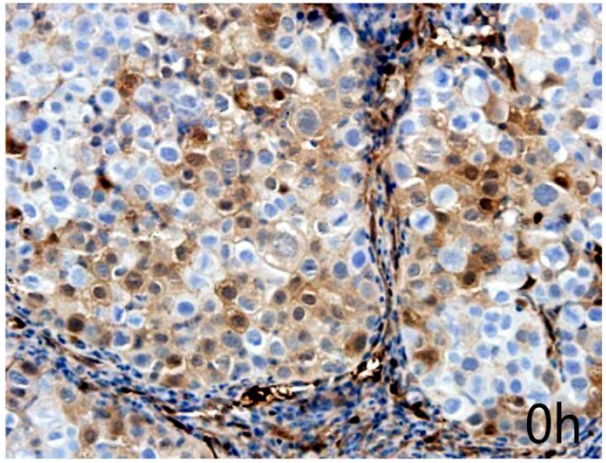
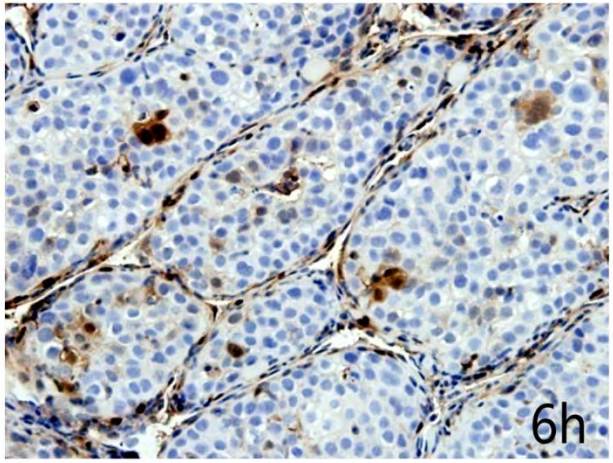
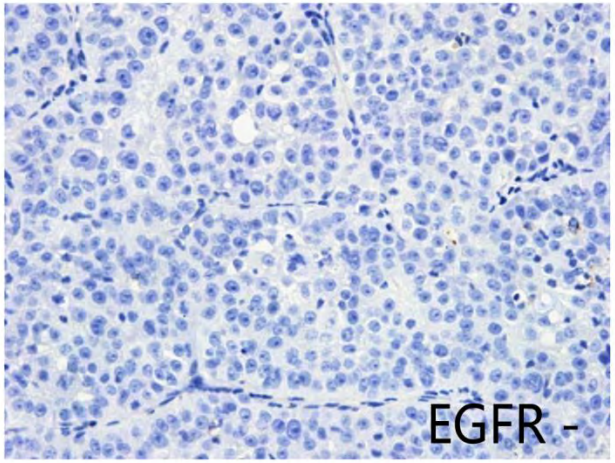
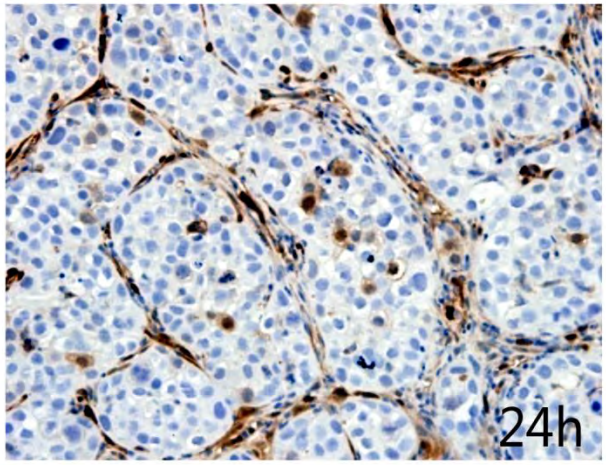
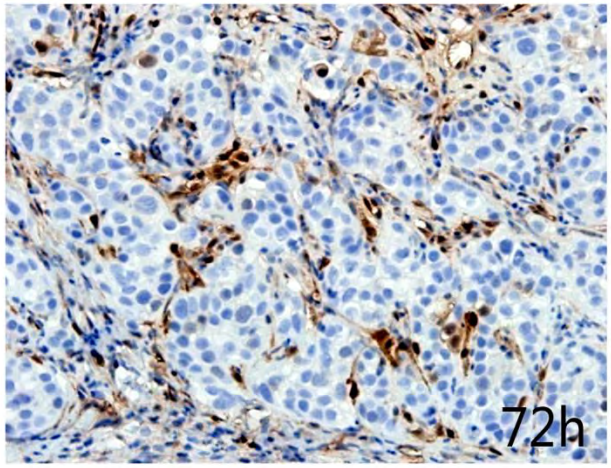
Cancer Biobank

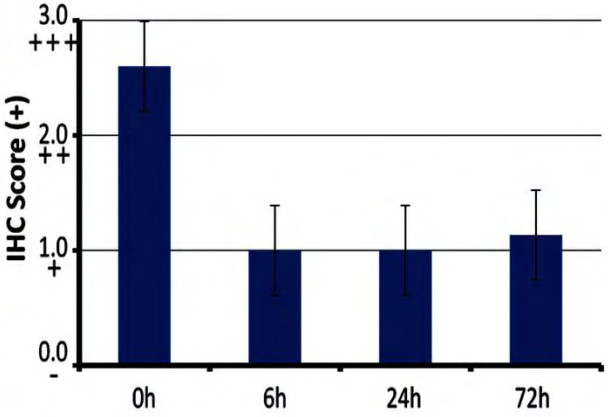












Response Prediction Biomarkers

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Cancer Biobank

Table 1. Treatment response, EGFR status and mutation status of GC PDX models.

Model ID	Non-Responders																Responders				*P* value (non- vs. responders)
	GA 0114	GA 2140	GA 0006	GA 0119	GA 0139	GA 0138	GA 0037	GA 0033	GA 0023	GA 0080	GA 0151	GA 0044	GA 0098	GA 0060	GA 0055	GA 0025	GA 0022	GA 0046	GA 0075	GA 0152	
ΔT/ΔC	1.744	1.492	1.42	0.934	0.912	0.898	0.881	0.811	0.781	0.748	0.717	0.687	0.58	0.488	0.41	0.305	-0.071	-0.078	-0.098	-0.121	0.002
Copy Number																					
EGFR (SNP6 + PICNIC)	6	NE*	7	5	5	5	5	5	4	6	NE	5	5	5	5	NE	7	7	8	15	0.002
EGFR (q-PCR)	5.1	3.8	2.1	2.1	4.8	2.7	3.7	3.9	1.9	3.7	1.4	2.1	3.7	3.1	2.9	2.1	5.4	4.3	4.6	1040.9	0.008
EGFR (FISH)	1.9	2.3	2.0	2.0	3.0	2.6	2.7	2.4	2.1	2.4	2.1	2.4	2.5	2.0	2.0	2.4	2.8	2.3	5.8	>15	0.029
CEP7 (FISH)	1.9	2.2	2.1	2.0	2.7	2.0	2.3	2.0	2.5	2.1	2.2	2.3	2.1	2.2	2.0	2.3	2.0	2.3	5.2		
Ratio (EGFR/CEP7)	0.96	1.04	0.97	1.03	1.09	1.29	1.16	1.21	0.83	1.16	0.93	1.03	1.2	0.91	1.01	1.05	1.39	1.03	1.12	>15	0.099
mRNA																					
EGFR U219 intensity	2.9	NE	3.3	3.6	2.9	2.3	2.5	2.4	2.5	2.8	3.6	3.1	4.3	4.2	4	3.8	6.5	6.9	5.8	10.5	0.003
EGFR Relative Intensity	0	0.1	0.08	0.14	0	0	0	0.02	0.02	0.02	0.2	0.13	0.07	0.13	0.1	0.1	0.81	0.62	0.5	13	0.002
Protein																					
EGFR IHC Score	0	2	1	1	0	0	0	0	0	1	1	1	1	1	2	0	3	3	3	3	0.002
Mutation																					
EGFR | Exon18;19;20;21	WT	NE	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	
k-RAS | Exon2;3;4	WT	NE	WT	WT	G13D	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	
BRAF | Exon15	WT	NE	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	
c-MET | Exon14;16;17;18;19;21	WT	NE	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	
PIK3CA | Exon1;9;20	WT	NE	WT	WT	WT	WT	WT	WT	WT	WT	WT	Del* 327-329	G545Y	WT	WT	WT	WT	WT	WT	WT	

These model are sorted by the ratio of ΔT/ΔC, and the final four are responders.* Del: deletion; NE: not evaluable.

SCIENTIFIC REPORTS

Response Prediction Biomarkers

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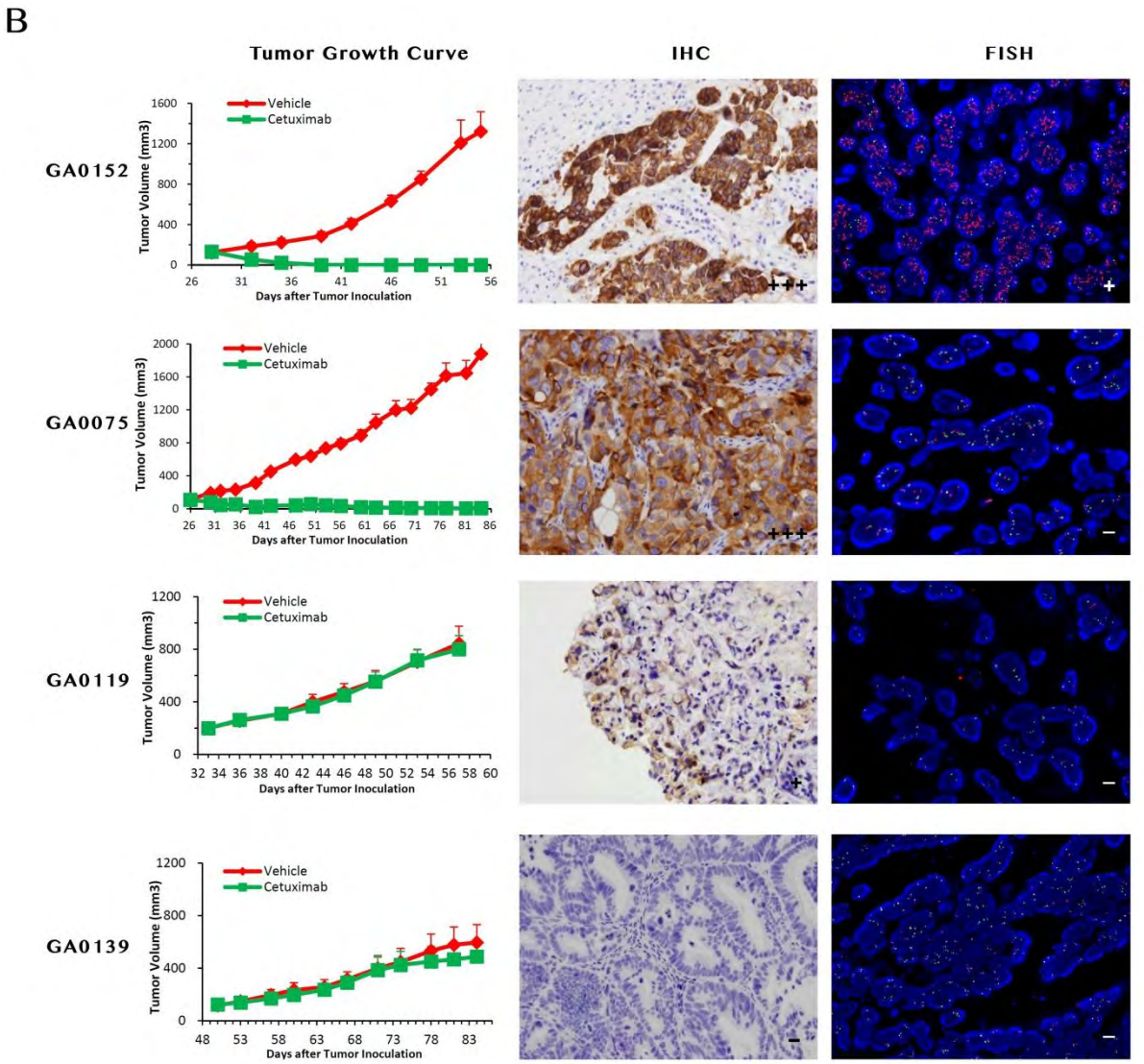

Response Prediction Biomarkers: EGFR DNA Copy number and RNA expression

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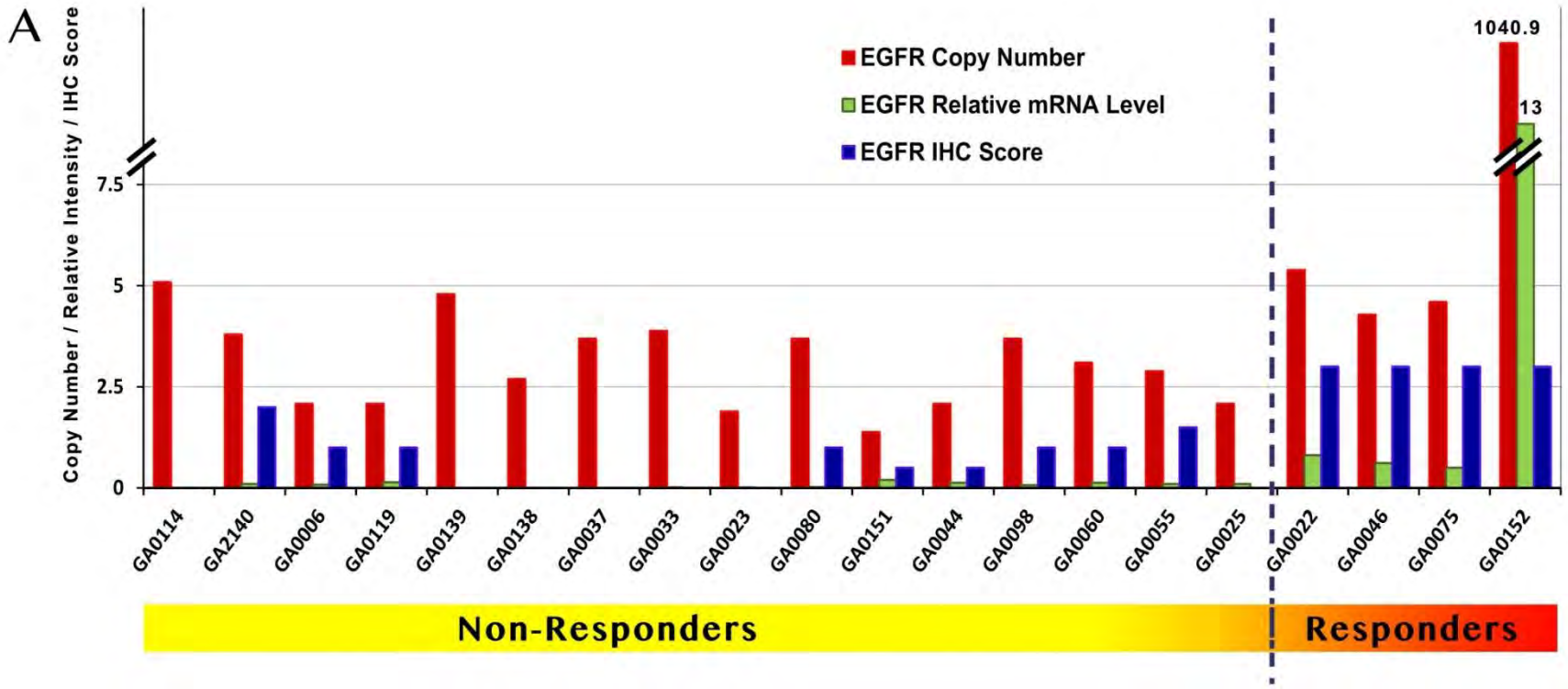
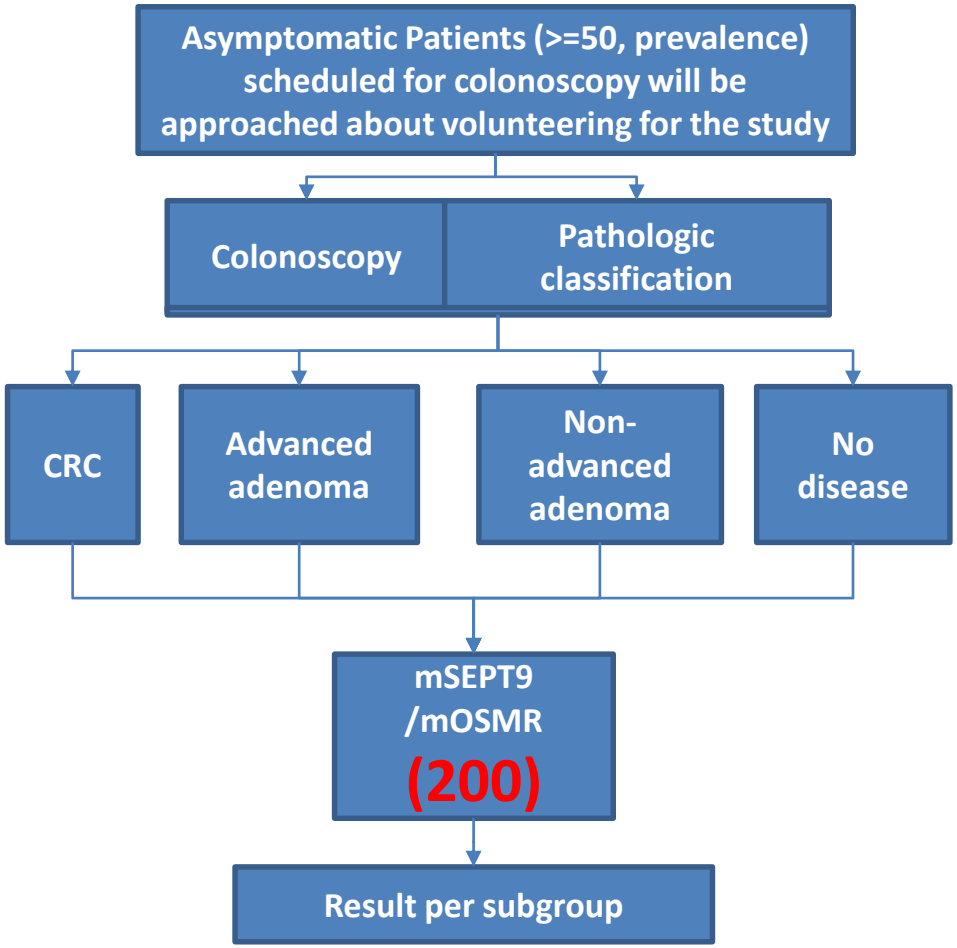


SCIENTIFIC REPORTS

③ DIAGNOSTIC TRIAL

PEKING UNIVERSITY CANCER HOSPITAL Cancer Biobank

Assessed the accuracy of Circulating Biomarkers for detecting CRC in a screening population.



7941 enrolled. 53 CRC cases a standardised sensitivity of 48.2%; for CRC stages I–IV, values were 35.0%, 63.0%, 46.0% and 77.4%, respectively. Specificity was 91.5%

Conclusions The blood based mSEPT9 test showed that CRC signal in blood can be detected in asymptomatic average risk individuals undergoing screening. Clinical Trial Registration Number: NCT00855348

GUT 2013 2012-304149

分享：诊断类临床研究计划

What is known

- Current CRC screening guidelines include FOBT or colonoscopy.
  - FOBT exhibits a low compliance rate and low sensitivity.
  - Colonoscopy is gold standard for detection, Which however has a due to its high cost and inadequate patient acceptance.

Pilot Study

- Detection of CRC in peripheral blood by Septin 9[1,2]/ OSMR[3] DNA methylation has been validated by our lab in Q-PCR based testing assay

Biomarker	Overall	I-II Stage	III-IV Stage	Volunteer
SEPT9	72.26%(99/137)	69.27%(54/78)	76.27%(45/59)	8.26% （9/109）
OSMR	91.23%(156/171)	91.67%（88/96）	90.67%（68/75）	13.8%（15/109）

1. PLoS One,2008:e3759
2. GUT 2013 2012-304149
3. Clin Cancer Res 2009:15,519

建议：

中华流行病学杂志 2006 年 10 月第 27 卷第 10 期

如何撰写高质量的流行病学研究论文

诊断试验准确性研究的报告规范——STARD 介绍

王波　詹思延

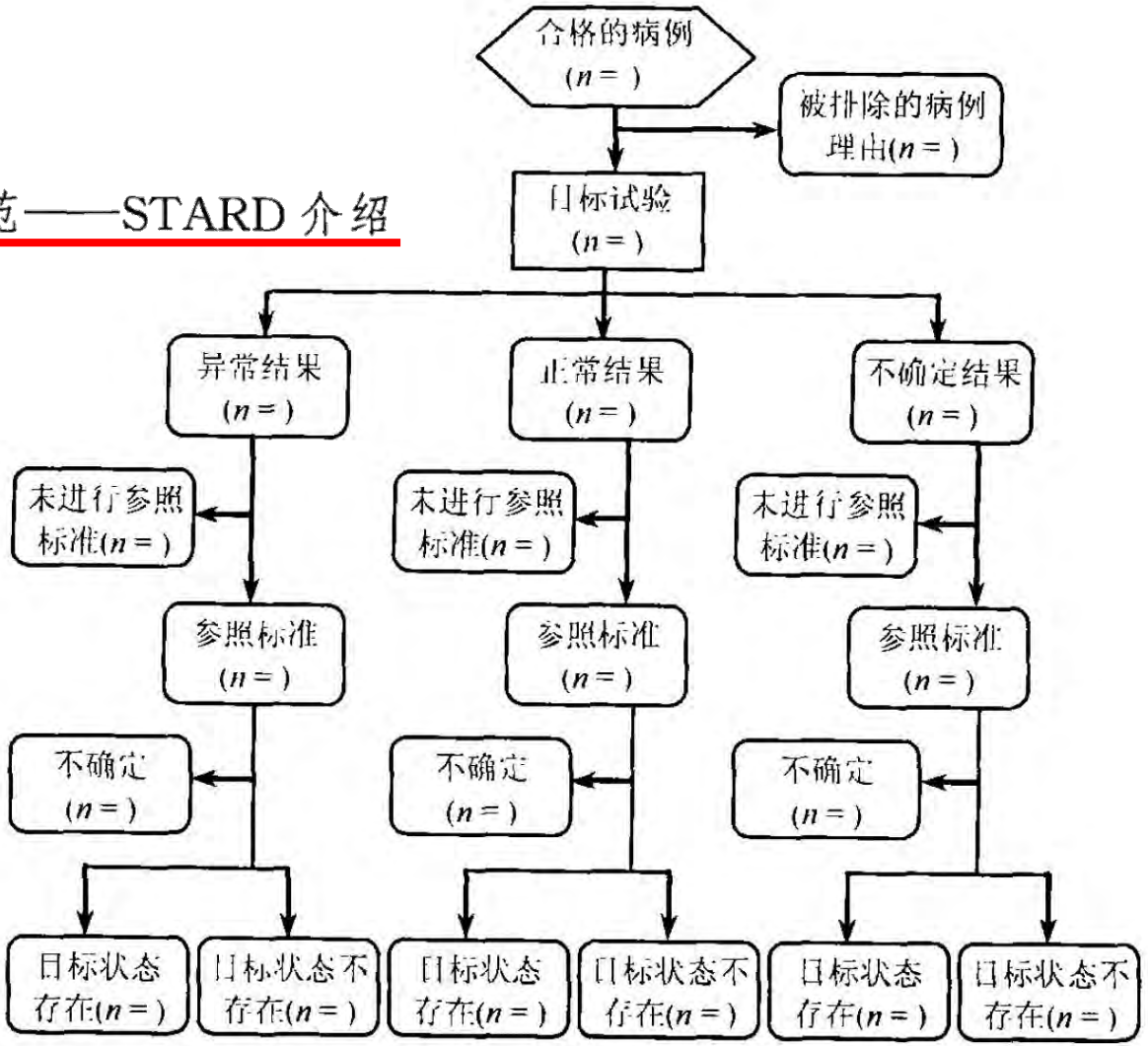


图1　诊断准确性研究流程图

样本库支持的项目

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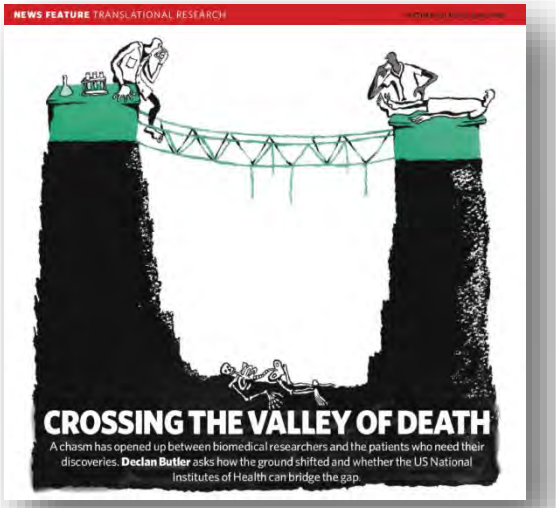
- Clinical Trial
- Pre-clinical Trial
- Basic Research
- Diagnostic Trial
- Biobank Infrastructure Construction



RESOURCES SHARING FOR LARGE PROJECT

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- Very urgent demand to share samples for important researches: ethics, standardization…

National Cancer resource network

- Even globe…

小结

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CANCER HOSPITAL
Cancer Biobank

1. 样本库是转化医学研究的核心工程

2. 设计良好的临床研究+保存良好的样本，将有助于实现有临床应用价值标志物的发现。

3. 联合起来力量大。

谢谢！

Thanks for your attention!

Lianhai Zhang, M.D.
Peking University Cancer Hospital & Institute
Email: zlhzlh@hotmail.com