資料 2

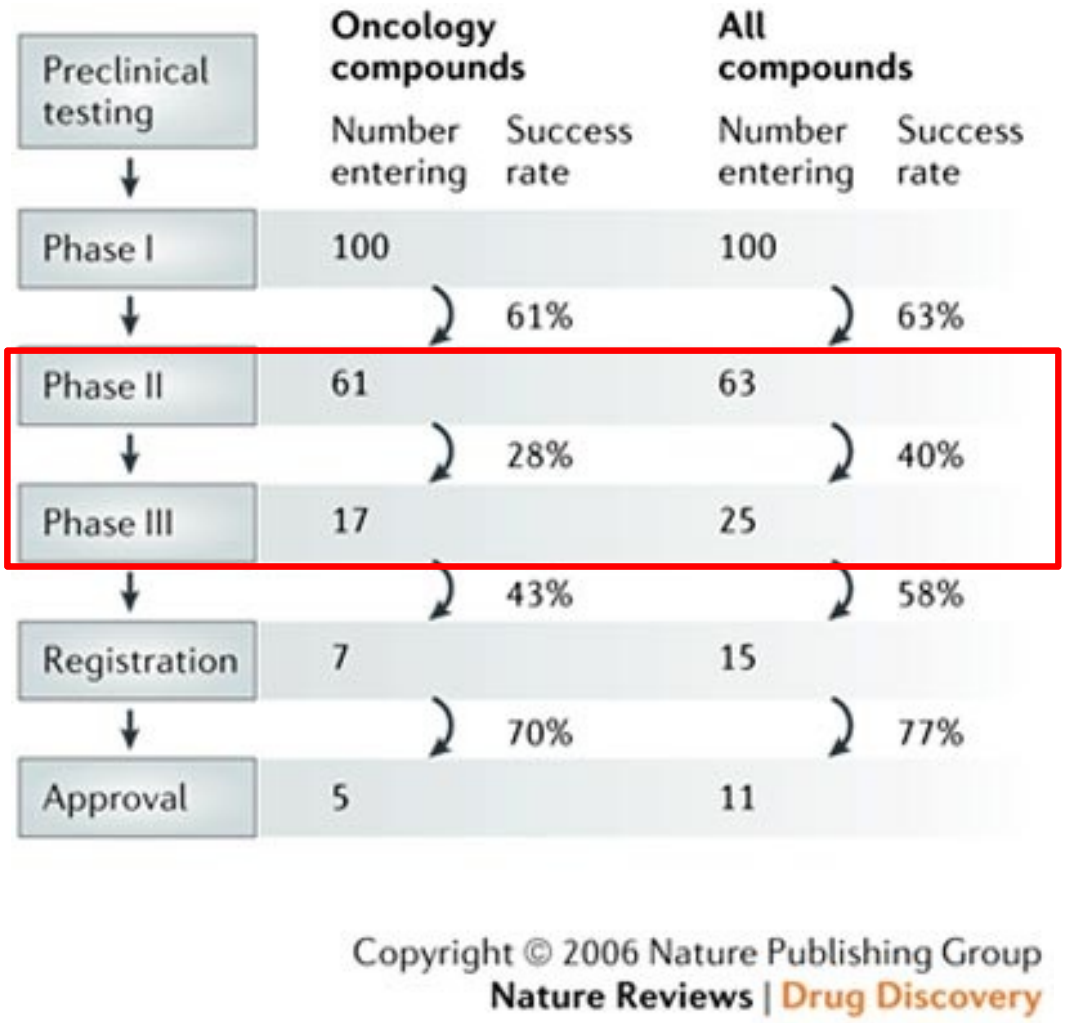
2014年12月18日
「非臨床試験の活用に関する専門部会」
第2回専門部会

抗がん剤の開発における薬効評価モデル系

早川　芳弘

富山大学　和漢医薬学総合研究所　病態生化学分野

Oncology領域での創薬プロセスにおけるPhase IIの壁



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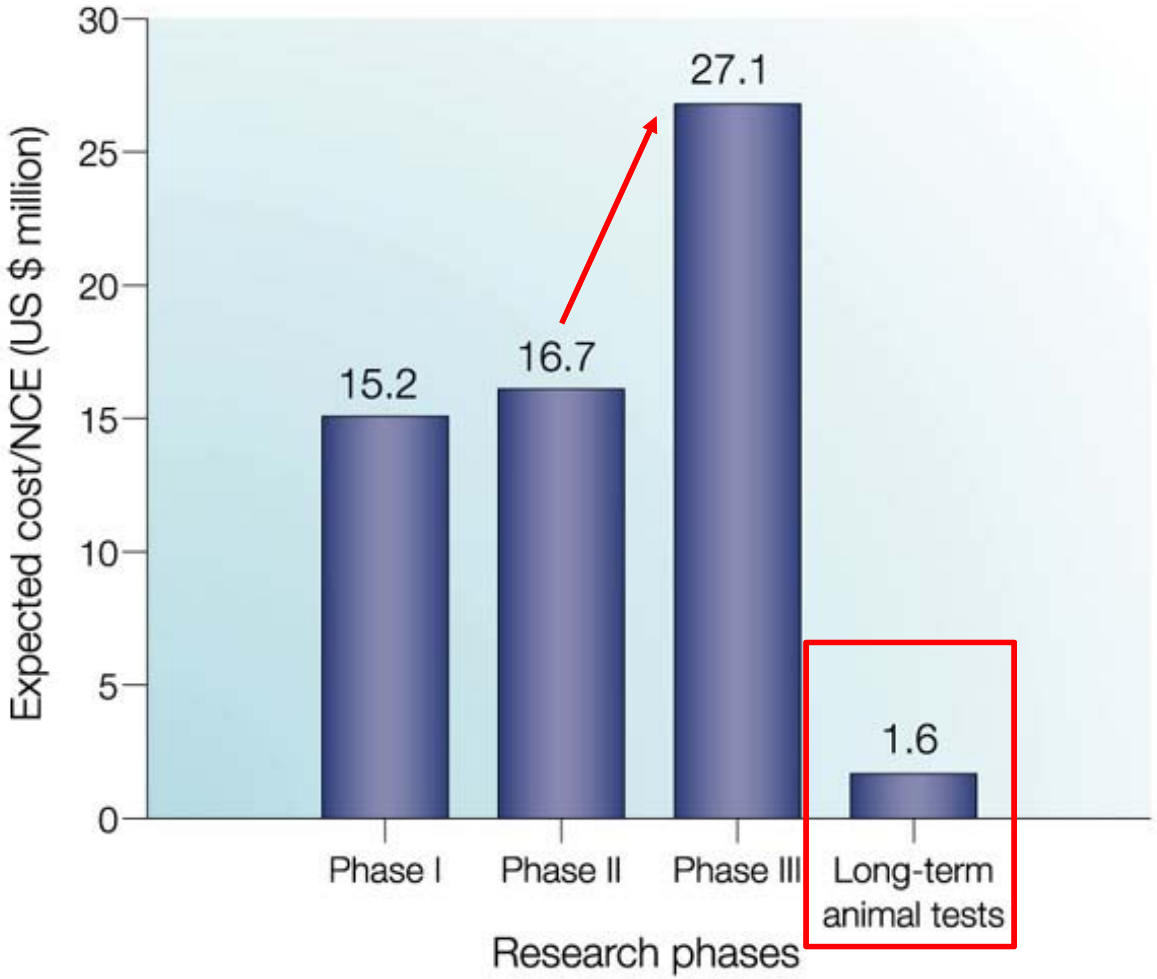
数多くの開発候補品がPhase II から III へ進めない

臨床でのPOC獲得の難しさ

Oncology領域での創薬プロセスにおけるPhase IIの壁



POS向上に向けた前臨床試験の役割

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前臨床試験で用いられるがんモデル

モデルの複雑さ

低

高

技術、コスト、時間

簡単

難しい

異種移植（ゼノグラフト：Xenograft）モデル
ヒトがん細胞
⇒異種（xeno）移植(免疫不全動物)

同種移植モデル
（主に）げっ歯類（マウス）がん細胞
⇒同種移植

同所性移植モデル
がん細胞 ⇒ がん細胞由来の組織、または転移標的臓器への移植

化学発癌モデル
⇒発癌物質（イニシエーター／プロモーター）による発がん誘導
主に変異原性により誘発される

遺伝子改変動物モデル
⇒特異的な遺伝子欠損／発現による発がん誘導

移植モデル

ヒトがん細胞を用いた異種移植モデル

マウスがん細胞を用いた同種移植モデル

臨床での薬効の予測には必ずしも役立っていないとの指摘

http://www.bjcancer.com

Relationships between drug activity in NCI preclinical in vitro and in vivo models and early clinical trials

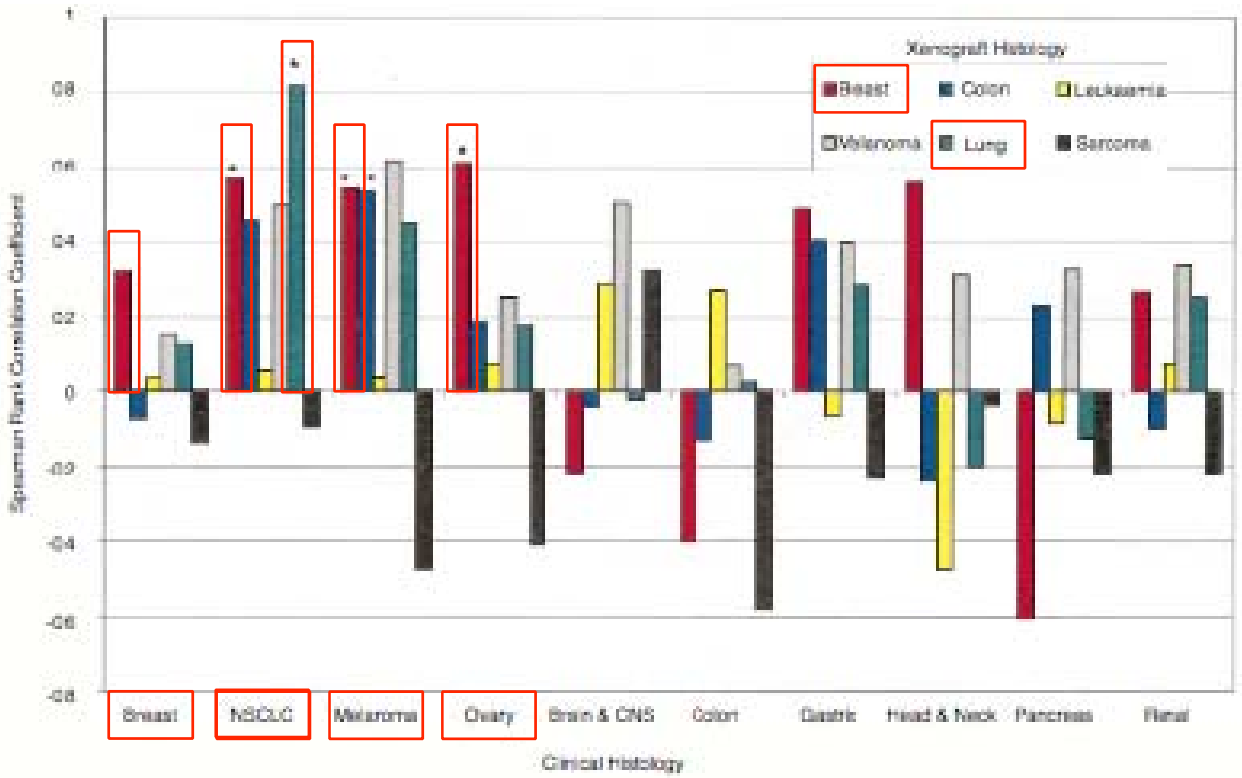
JI Johnson,[1] S Decker,[1] D Zaharevitz,[1] LV Rubinstein,[2] JM Venditti,[3] S Schepartz,[1] S Kalyandrug,[2] M Christian,[2] S Arbuck,[3] M Hollingshead[1] and EA Sausville[1]

[1]Developmental Therapeutics Program, [2]Cancer Therapy Evaluation Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Bethesda, MD 20892, and [3]SAIC-Frederick, Frederick, MD 21701

Table 1 NSC numbers and common names for 39 phase II clinical agents

NSC Number	Common name
740	Methotrexate
3053	Actinomycin-D
3088	Chlorambucil
8806	Melphalan
19893	5-FU
26271	Cyclophosphamide
26980	Mitomycin C
45388	Dacarbazine
49842	Vinblastine
105014	2-CDA
119875	Cisplatin
123127	Adriamycin HCL
125066	Bleomycin
125973	Paclitaxel
141633	Homoharringtonine
172112	Spiromustine
253272	Caracemide
264880	Dihydro-5-azacytidine
267469	Deoxydoxorubicin
269148	Menogaril
281272	Fazarabine
286193	Tiazofurin
308847	Amonafide
312887	Fludarabine phosphate
325319	Didemnin B
332598	Rhizoxin
336628	Merbarone
337766	Bisantrene
339004	Chloroquinoxaline sulfonamide
347512	Flavone acetic acid
349174	Piroxantrone hydrochloride
352122	Trimetrexate
356894	Deoxyspergualin
361456	Pyrazine diazohydroxide
366140	Pyrazoloacridine
409962	BCNU
609699	Hycamptamine
616348	CPT-11 (irinotecan)
628503	Taxotere (docetaxel)

前臨床試験と臨床試験の結果における相関関係



OPINION

What's wrong with our cancer models?

Alexander Kamb

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doi:10.1038/nrd1635

When cancer models work

To arrive at a firm conclusion about the deficiencies of tumour models as they are conventionally used, it is sensible to explore cases in which such models are predictive. This yields a remarkably simple insight: the single most important determinant of success is the degree to which the cell models match the genetic traits of the real malignancy to be treated in the clinic. Indeed, there is a small but increasing number of drugs for which preclinical models accord with clinical success (TABLE 4). These models involve tumours that contain recurrent mutations, amplifications or translocations, including chronic myelogenous leukaemia (CML) resulting from BCR–ABL fusions; gastro-intestinal stromal tumour (GIST) resulting from mutations in KIT; lung adenocarcinoma resulting from mutations in the epidermal growth factor receptor (EGFR); acute myelogenous leukaemia (AML) resulting from mutations in FLT3; and breast carcinoma resulting from amplifications of the HER2/*neu* gene. Drugs that target ABL, KIT, EGFR, FLT3 and HER2/*neu* work well in tumours that contain, respectively, activated BCR–ABL fusions[9], mutant KIT[10], EGFR[11] and FLT3[12] and amplified HER2/*neu*[13]. Cultured tumour cells and cell lines derived from tumours with these genetic hallmarks consistently display increased sensitivity to the cognate inhibitors: imatinib (Glivec; Novartis) for CML and GIST; gefitinib (Iressa; AstraZeneca) and erlotinib (Tarceva; OSI/Genentech/Roche) for lung cancer; PKC412 and other investigational drugs for AML; and trastuzumab (Herceptin; Genentech/Roche) for breast cancer. Mouse models developed on the basis of lines derived from tumours with the relevant translocations or mutations also show dramatic responses, assuming that the drug has suitable pharmacokinetic properties in the animal. Genetic lesions therefore confer a benefit to the tumour, a differential dependency that is observed in the clinic and paralleled by cancer models that contain such lesions. These findings, limited as they are, prove the hypothesis that tumour models that carry the genetic signature of the native malignancy can recapitulate clinical behaviour.

Table 4 | **Drugs and preclinical efficacy/toxicity testing***

Drug	Target	Cell culture model	Mouse model
Glivec	BCR–ABL	Yes	Yes
Glivec	KIT	Yes	Yes[‡]
Iressa/Tarceva	EGFR	Yes	ND
Herceptin	HER2/neu	Yes	Yes
PKC412	FLT3	Yes	Yes

*Results look at shifts in dose–response between cell lines/xenografts containing recurrent mutation/translocation/amplification and others. ‡Mouse GIST wild-type Kit model also sensitive. Yes = dose–response shift compared with wild-type gene; ND = not done. EGFR, epidermal growth factor receptor.

ターゲットとする遺伝形質の重要性

OPINION

What's wrong with our cancer models?

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doi:10.1038/nrd1635

When cancer models fail

Extrapolation from small numbers of ill-characterized xenografts is fraught with risk. Cancer is a diverse disease. Researchers might survey a panel of xenografts and find one or more that is sensitive to a drug, but in general have no idea why a given cell line responds to the agent whereas another does not.

We should allow that the commonly used cancer models invariably add some value to drug development projects. Even if the tendency is to over-interpret the meaning of %T/C in xenograft models, it is helpful to learn about drug properties of the compound in animals; mundane but key features such as pharmacokinetic behaviour, tissue distribution, percentage of free (unbound) compound in plasma and so on can be determined in such models.

既存モデルでのPK/PD測定の有用性

A very tough question is whether the regulatory authorities should permit investigational drugs to be given to patients when the supporting preclinical evidence is suspect in the ways discussed above. The trend in Phase I oncology studies shows that treatment-related deaths have fallen to a fraction of a percentage point[4]. It can therefore be argued that the costs of testing compounds are principally financial, and not counted in human lives. In the absence of better models, it seems unwise to halt clinical trials that sometimes reveal benefits even when the preclinical rationale is not perfectly clear (such as, for example, proteasome inhibition). Nevertheless, a paradigm shift often requires a change to established processes. As such, we might expect that regulatory agencies will begin to insist on a clearer definition of preclinical models that represent the clinical population that will be best treated by a new drug. Progress toward this goal could require that we identify essential and compensating functions specific to cancer cells. We must therefore develop the tools and an analytic framework not only to resolve the genetic/epigenetic states of human cells, but also to untangle the crucial gene–gene interactions that affect therapeutic responses. The challenge of patient stratification therefore merges with the problem of choosing proper preclinical models.

前臨床モデルがどのような患者背景を表しているか理解する事が重要

Alternatives

A variant of the subcutaneous xenograft is the orthotopic model, typically a xenograft grown in proximity to the tissue type from which the tumour cell line originated. The normal milieu of a tumour, with its network of tumour–host interactions, provides a strong rationale for such models, and several examples of differences in the details of xenograft growth in orthotopic versus subcutaneous settings can be found. Overall, however, this approach seems to be a relatively modest improvement as a modelling strategy. When the targets under study clearly involve non-autonomous cellular behaviour such as angiogenesis, orthotopic models might be more predictive. But for robust, autonomous cellular functions, it is not clear that the tradeoffs of convenience and throughput are justified.

宿主ーがん細胞相互作用を前臨床試験においても考慮する事の重要性、宿主環境を考慮した移植モデルの意義

移植モデル

ヒトがん細胞を用いた異種移植モデル

ヒトがん細胞→免疫不全動物の皮下へ移植（異種異所性移植モデル）
→免疫不全動物の対応する癌細胞由来組織へ移植（異種同所性移植）
→免疫不全動物の脈管内へ移植（実験的転移モデル）

マウスがん細胞を用いた移植モデル

マウスがん細胞→同系マウスの皮下へ移植（同種異所性移植モデル）
→同系マウスの癌細胞由来組織へ移植（同所性移植モデル）
→同系マウスの脈管内へ移植（実験的転移モデル）

同所移植モデル

肝癌　　→　　肝臓
肺癌　　→　　肺
大腸／結腸癌　→　　(盲)腸
前立腺癌　　→　　前立腺
腎癌　　→　　腎臓
脳腫瘍　→　　脳
骨肉腫　→　　骨

（実験的）転移モデル

経尾静脈接種　→　主に肺（まれに肝臓）

門脈／経脾接種　→　肝臓

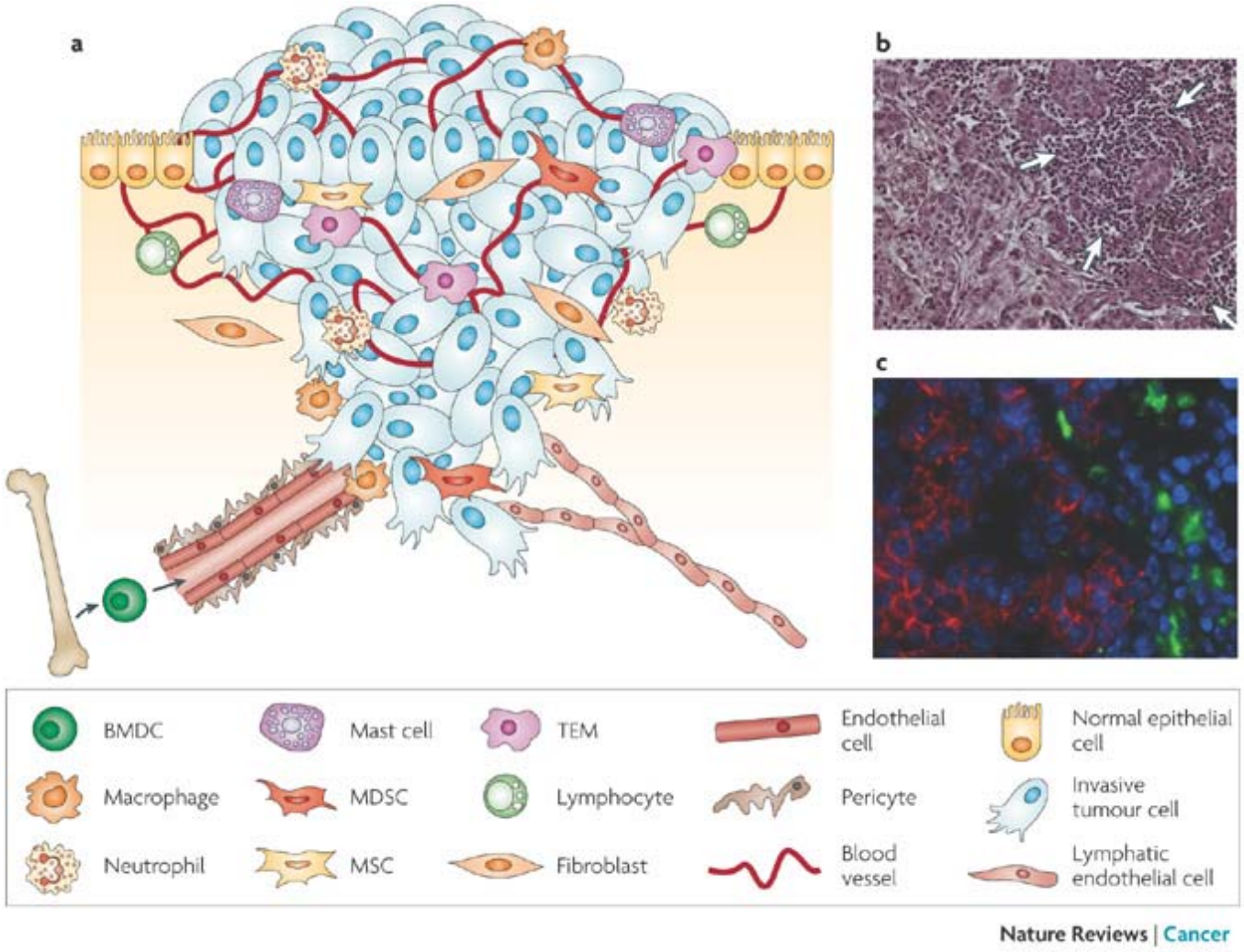
左心室内接種　→　全身性（骨を含む）

皮下接種（足せきを含む）　→　肺

乳腺接種　→　全身性

腫瘍微小環境

～腫瘍を取り巻く環境を含めてがん病態を捉える事が重要である～



Johanna A. Joyce & Jeffrey W. Pollard 2009
Nature Reviews Cancer 9, 239-252

腫瘍微小環境ーがん細胞相互作用が薬効評価に及ぼす影響

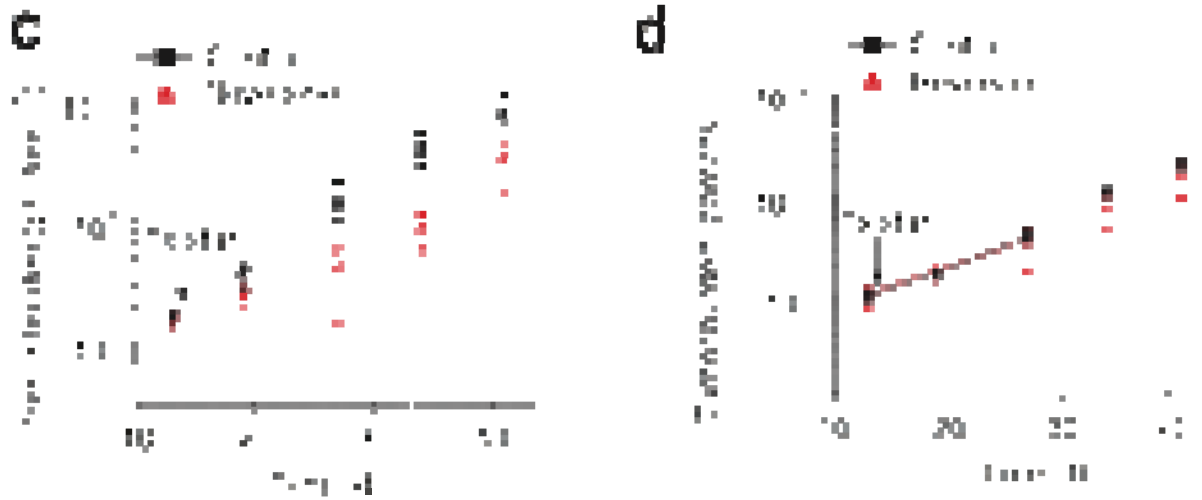
Tumor cell-specific bioluminescence platform to identify stroma-induced changes to anticancer drug activity

Douglas W McMillin[1–3], Jake Delmore[1–3], Ellen Weisberg[2,3], Joseph M Negri[1–3], D Corey Geer[1–3], Steffen Klippel[1–3], Nicholas Mitsiades[1,2], Robert L Schlossman[1–3], Nikhil C Munshi[1–3], Andrew L Kung[3], James D Griffin[2,3], Paul G Richardson[1–3], Kenneth C Anderson[1–3] & Constantine S Mitsiades[1–3]

NATURE MEDICINE

subcutaneous

intravenous (bone)



腫瘍微小環境ーがん細胞相互作用が薬効評価に及ぼす影響

LETTER

doi:10.1038/nature11183

Tumour micro-environment elicits innate resistance to RAF inhibitors through HGF secretion

Ravid Straussman[1], Teppei Morikawa[2], Kevin Shee[1], Michal Barzily-Rokni[1], Zhi Rong Qian[2], Jinyan Du[1], Ashli Davis[1], Margaret M. Mongare[1], Joshua Gould[1], Dennie T. Frederick[3], Zachary A. Cooper[3], Paul B. Chapman[3], David B. Solit[3], Antoni Ribas[3,4], Roger S. Lo[4,5], Keith T. Flaherty[3], Shuji Ogino[2,6], Jennifer A. Wargo[3] & Todd R. Golub[1,7,8]

LETTER

doi:10.1038/nature11249

Widespread potential for growth-factor-driven resistance to anticancer kinase inhibitors

Timothy A. Wilson[1], Jane Karnezis[1], Yibing Yan[1], Elena Nemazanyy[1], Luciana Barbon[1], Emily Char[1], Jing Peng[1], Eva Lin[1], Yulei Wang[1], Jeff Settman[1], Antoni Ribas[2], Jiang Li[1], John Moffat[1], Daniel P. Sutherlin[3], Hartmut Koeppen[1], Mark Merchant[1], Richard Neve[1] & Jeff Settleman[1]

腫瘍微小環境ーがん細胞相互作用が薬効評価に及ぼす影響

The role of tumour–stromal interactions in modifying drug response: challenges and opportunities

Douglas W. McMillin[1–4], Joseph M. Negri[2] and Constantine S. Mitsiades[1,3]

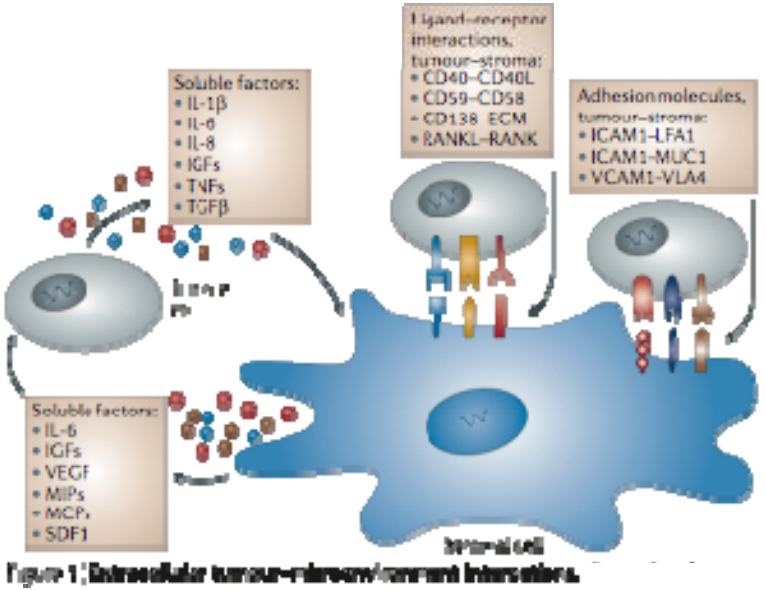
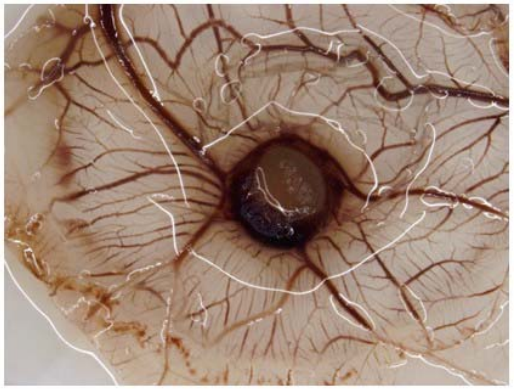


Table 1 | Examples of therapeutic agents that are subject to stroma-induced drug resistance in preclinical models[a]

Therapeutic agents	Tumour type	Impact of tumour–stromal interactions on preclinical antitumour activity	Clinical observations
Dexamethasone	Multiple myeloma	Stroma-induced resistance[35]	Randomized clinical trial[139] showed inferior clinical outcome compared to bortezomib, a proteasome inhibitor that overcomes stroma-induced resistance in preclinical models of multiple myeloma
Doxorubicin, melphalan	Multiple myeloma	Stroma-induced resistance[35]	Potent in vitro activity of doxorubicin against multiple myeloma cell monocultures[35], but limited single-agent clinical activity at conventional doses[140]; myeloablative melphalan doses are used as conditioning regimen prior to autologous stem cell transplantation, but are not curative for multiple myeloma
Doxorubicin	Anaplastic thyroid carcinoma	Stroma-induced resistance[23,141]	Limited, if any, responses of most patients to anthracycline-based chemotherapy[142]
Vemurafenib (PLX4032)	*BRAF*^{V600E}-mutant melanoma	Resistance induced by skin fibroblasts or their conditioned media[18,19]	Marked, but typically partial, responses to vemurafenib; frequent recurrence within 6 months of treatment[143–146]
Ruxolitinib (INCB018424)	*JAK2*^{V617F}-mutant myeloproliferative disorders	Resistance induced by conditioned media of bone marrow stromal cells[21]	Palliative clinical effect of ruxolitinib (partial clinical response in terms of decrease in the size of the spleen; alleviation of constitutional symptoms), but typically no histopathological, cytogenetic or molecular remissions; lack of survival advantage over the best available therapy[147]

腫瘍微小環境をターゲットとした実験系

血管新生：Angiogenesis



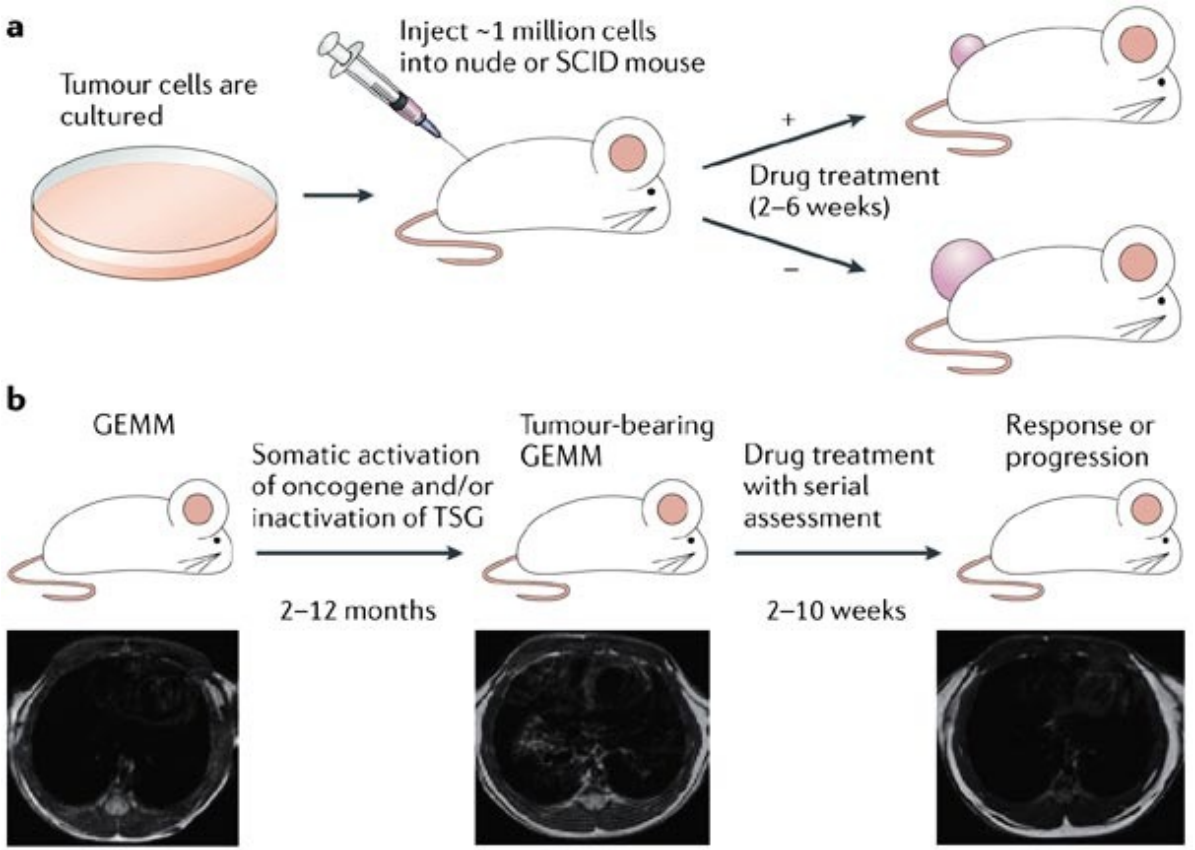
Chick Chorioallantoic Membrane (CAM) Assay

Hollow Fiber

マトリゲルプラグ

遺伝子改変動物モデル

GEMM: Genetically Engineered Mouse Model



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遺伝子改変動物モデル

GEMM: Genetically Engineered Mouse Model

Purpose	Examples of discoveries or application of knowledge gained from GEMMs	Refs
Target validation	p53 mediates the apoptotic effects of DNA-damaging agents	77
	K-RAS is not the anticancer target of FTIs	78
	CDK2 is not required for cellular proliferation	70,71
	PPARγ is not required for colorectal carcinogenesis	17
Therapy trials (in particular, these show regression of established tumours)	EGFR inhibitors against EGFR-mutant lung cancer	65,67
	Rapamycin in PTEN-mutant leukaemia and AKT/MYC-induced lymphoma	81,82
	Combination therapy with VEGFR and PDGFR inhibition in pancreatic islet carcinoma	79
Biomarker discovery (for example, to predict response, progression or subclinical disease)	In progress. Several unbiased efforts to find biomarkers to predict use in cancer screening are underway using GEMMs. However, biomarker discovery is a difficult process; for example, only one serological biomarker — prostate-specific antigen — is currently widely used for cancer screening.	–
Modelling resistance	bFGF-mediated escape from prolonged VEGFR blockade	84
	Metronomic therapy with angiogenesis inhibitors	80
Understanding toxicity	Predicting cardiac toxicity of HER2/*neu* inhibitors	96
	Predicting gastrointestinal and cutaneous toxicity of EGFR inhibitors	104
	Predicting less than expected toxicity from inhibition of specific CDKs (for example, CDK4)	105,106
	Predicting depigmentation from KIT inhibition	107

bFGF, basic fibroblast growth factor; CDK, cyclin-dependent kinase; EGFR, epidermal growth factor receptor; FTIs, farnesyltransferase inhibitors; GEMMs, genetically engineered mouse models; PDGFR, platelet-derived growth factor receptor; PPARγ, peroxisome proliferator-activated receptor-γ; PTEN, phosphatase and tensin homologue deleted on chromosome 10; VEGFR, vascular endothelial growth factor receptor.

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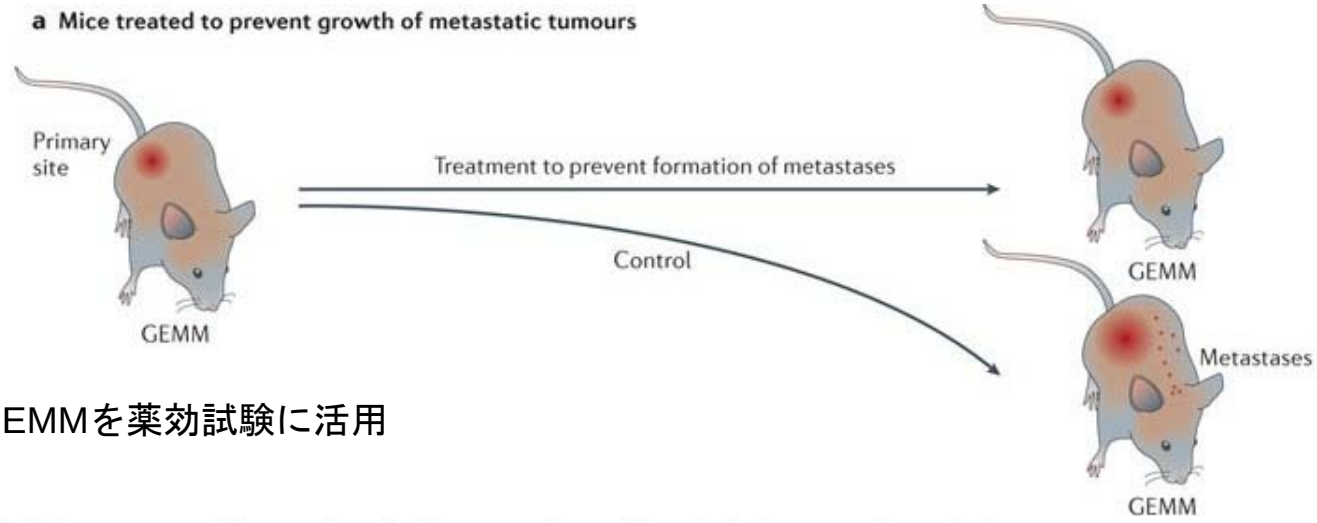
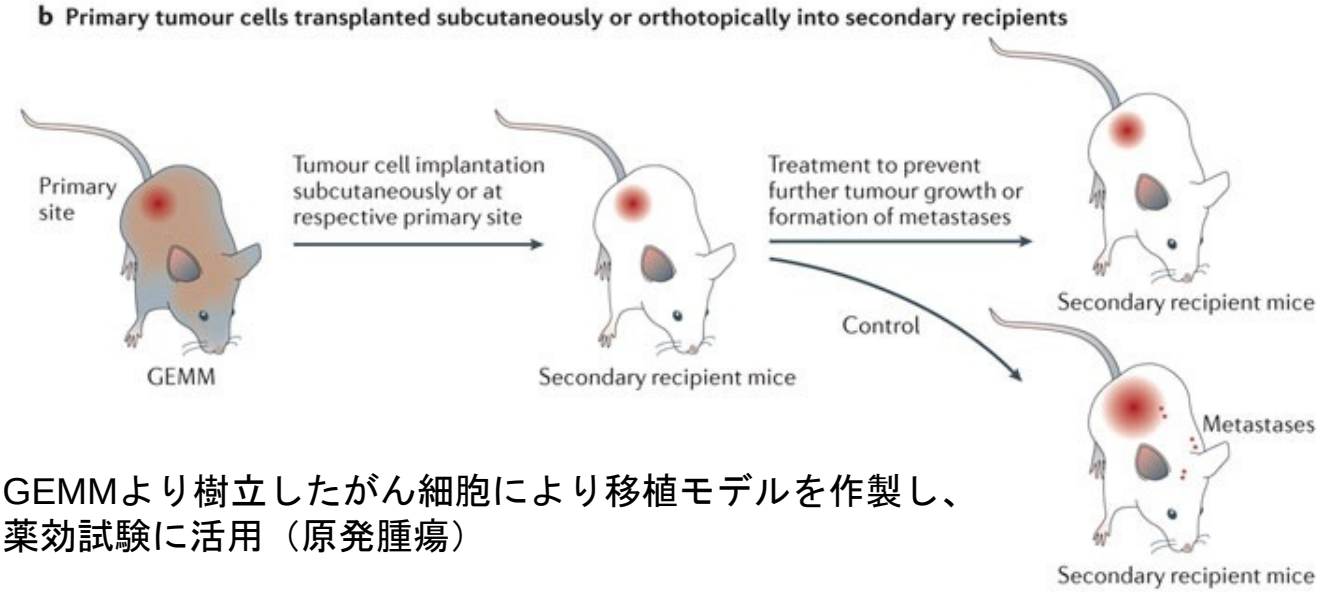
GEMMの応用例



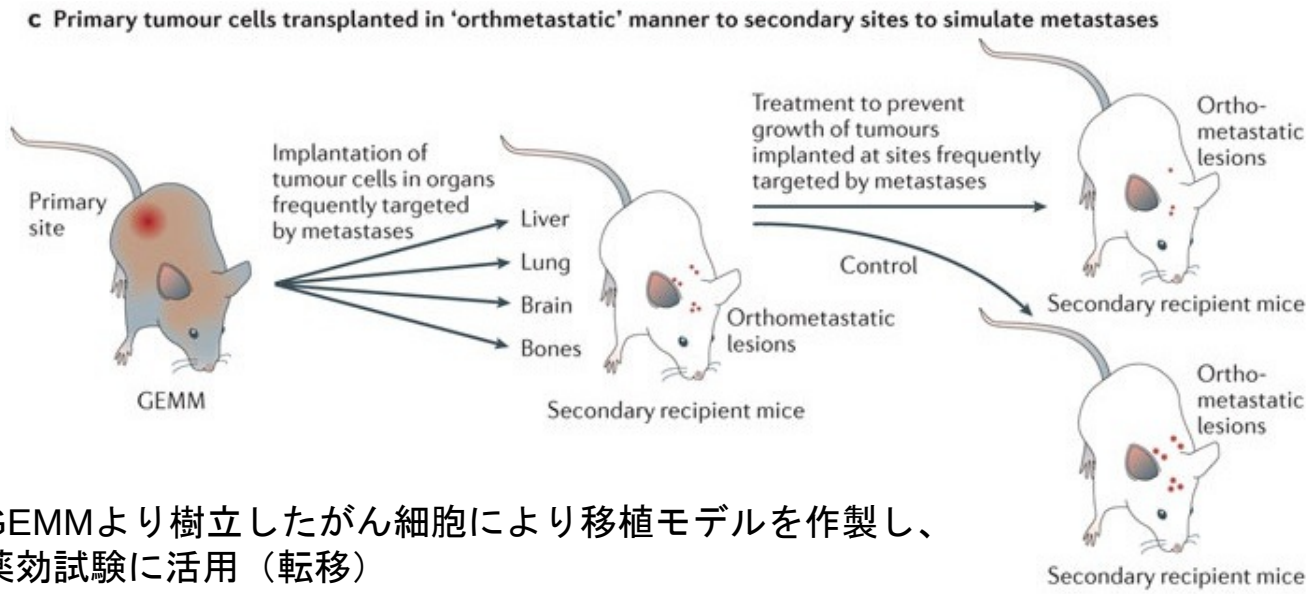
GEMMを薬効試験に活用



GEMMより樹立したがん細胞により移植モデルを作製し、
薬効試験に活用（原発腫瘍）

McMillin et al. Nature Reviews Drug Discovery (2013) 12, 217-228

GEMMの応用例



GEMMより樹立したがん細胞により移植モデルを作製し、
薬効試験に活用（転移）

McMillin et al. Nature Reviews Drug Discovery (2013) 12, 217-228

前臨床試験で用いられるがんモデル評価系

✦ 形態学的評価

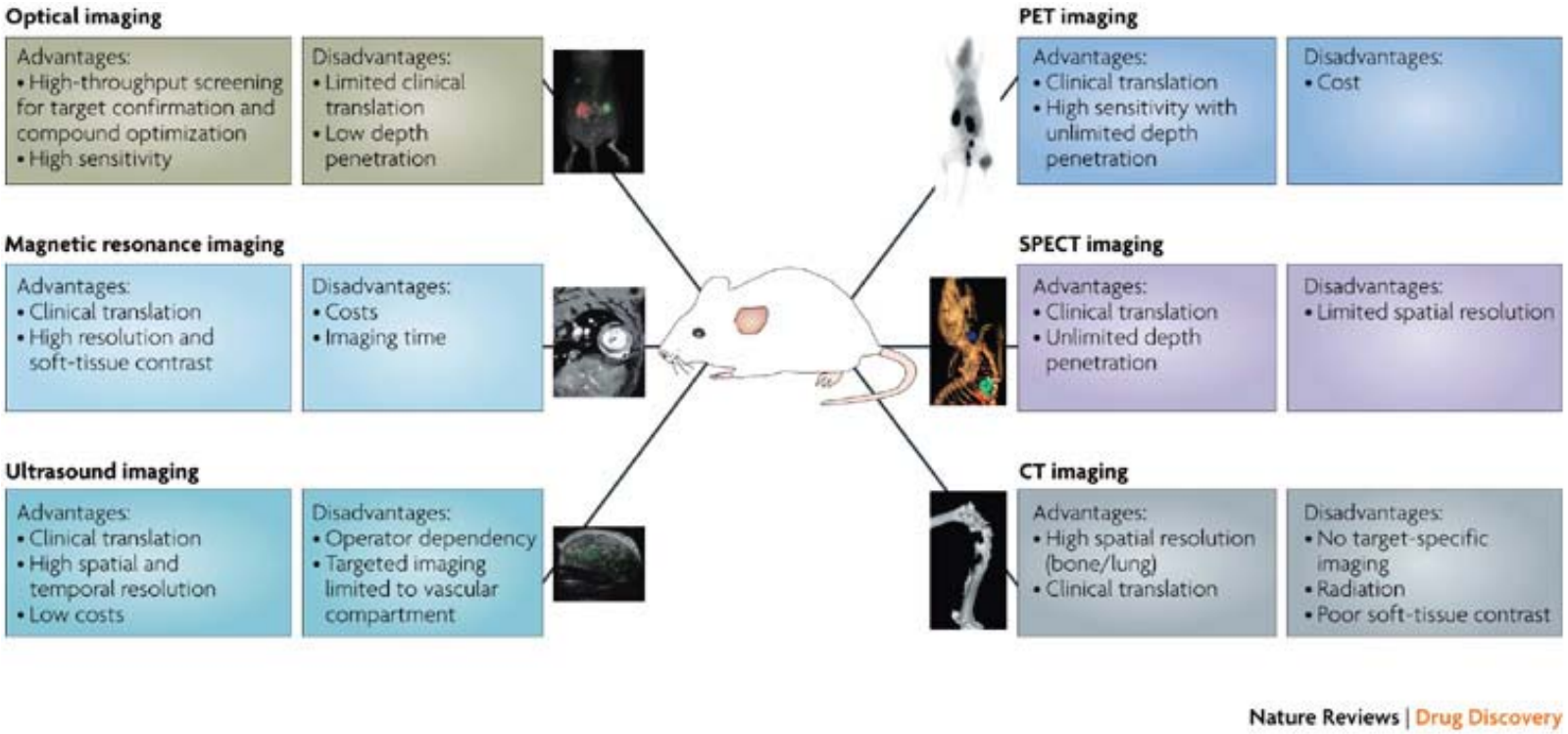
1. 腫瘍サイズの測定（腫瘍径、重量の測定）
2. 肉眼的所見（コロニー数、播種の有無、リンパ節腫脹など）

✦ 病理学的評価

✦ PK／PD測定

1. 標的ターゲット分子の腫瘍組織での発現（組織病理、遺伝子発現、タンパク発現、細胞レベルでの解析など）
2. 腫瘍組織での効果予想因子の発現
3. 血中レベルでの効果予想因子（代謝産物、タンパク発現など）
4. がん種に特異的な増殖因子／腫瘍マーカー

インビボイメージングによる評価



GEMMをはじめとするより臨床病態に近いと考えられる非常に複雑なマウスモデルの評価系としての活用

がん移植モデルの特徴

1.　がん細胞株の皮下移植による異所性移植モデル

- ターゲットに顕著な腫瘍増殖抑制作用が認められる場合、腫瘍移植の生着・増殖の判定・モニターが容易であること。
- ターゲットとその他の治療、薬剤との併用による効果の判定が比較的容易であること。
- 腫瘍組織サンプル採取などによる効果の判定も比較的容易に行えること。

2.　同所性移植モデル

- ほとんどの上皮系組織由来のがん細胞にとって皮下は異所組織であるため、特にヒト細胞株においてはそれぞれの臓器由来のがん細胞の特性をin vivoにおいて反映していない可能性が高い。
- 本来の発生臓器（または転移標的臓器）に移植する同所性移植は上述の点においてより適切であると考える。

がん移植モデルの特徴

3.　実験的転移モデル

- 原発巣を遊離したがん細胞が血管内に侵入するまでのステップをスキップし、がん細胞が脈管内に侵入した以降の過程を調べる事が可能なモデル。
- 簡便さ、再現性の高さおよび評価期間が比較的短い利点。
- 実際のがん転移における原発巣からの血管内侵入を想定すると10^4～10^5の癌細胞が脈管内に侵入する事は考えにくく、人工的な系であると言わざるを得ない。

4.　自然転移モデル

- 同所性または異所性に移植したがん細胞株による原発巣の形成・増殖から、転移形成の主な病態の過程をすべて反映する。
- 非常に理想的なモデルではあるが、自然転移するがん細胞株は非常に限られており、また実験全体における結果のばらつきが多いのも特徴。

GEMMの特徴

- 発がん／がんの悪性化に関連する分子の発現調節によりがん病態を形成することが出来る
- がん病態で起こる様々な生体内でのイベントを反映しうる
- 簡便さ、再現性、評価期間の観点からは移植モデルと比較して不利な点が多い
- がん病態のモニタリングが一般的に非常に困難である（すなわち開発品や新規治療法の効果判定が難しい）

分子標的が明確でない開発候補の薬効評価系

Phenotypic screening in cancer drug discovery — past, present and future

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1999年から2013年までの表現系スクリーニングによる創薬（oncology領域)

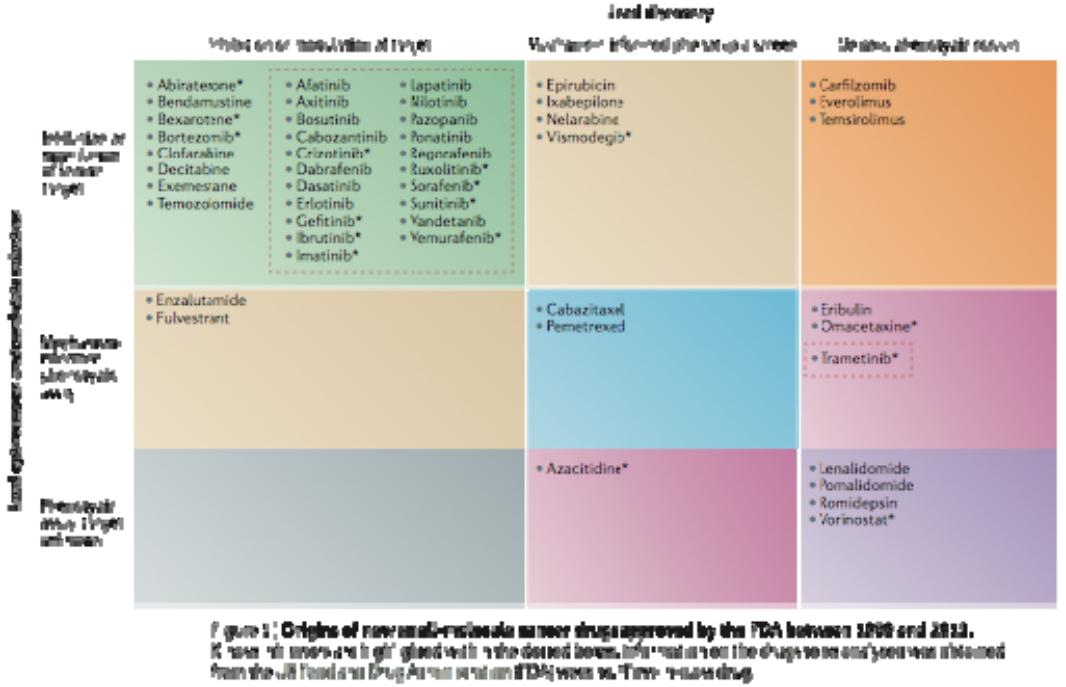
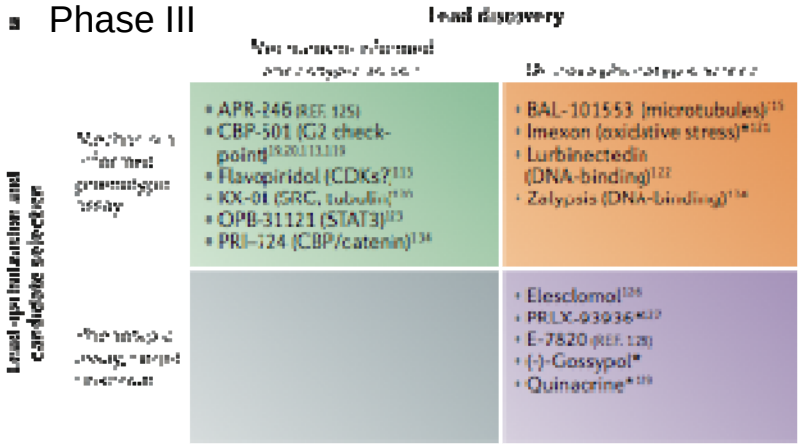


Figure 1 | Origins of new multi-molecule cancer drugs approved by the FDA between 1999 and 2013.



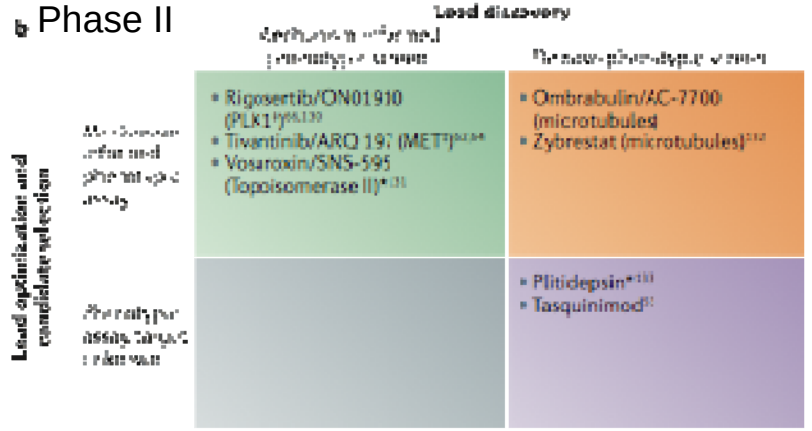


Figure 3 | Origins of phenotypically discovered cancer drugs currently in Phase III and Phase II trials. a | Drugs in Phase III trials. b | Drugs in Phase II trials. The highest

分子標的が明確でない開発候補の薬効評価系

NATURE REVIEWS DRUG DISCOVERY

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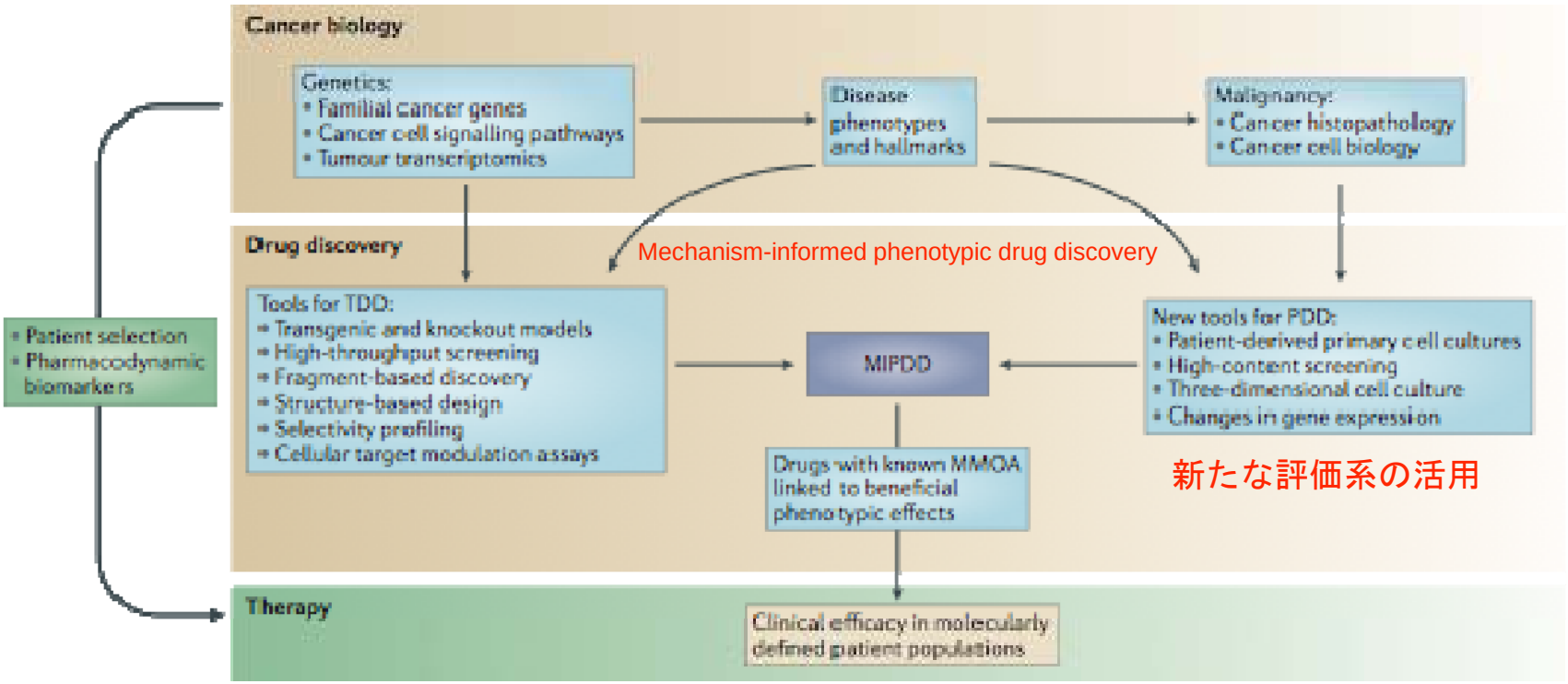

Figure 5 | A comparison between target-based drug discovery and phenotypic drug discovery, showing the linkage with mechanism-informed phenotypic drug discovery. The figure illustrates how the components

今後の議論の課題

- In vitroからin vivoへのtransitionの必要性（ヒトとマウス）
- In vitroで薬効が確認出来ているがん細胞を免疫不全動物に移植して評価することの意義（in vivo culture?）
- Xenograftモデルの結果がもたらす意義
- 新しい細胞培養系と比較した動物実験の意義
- 複雑なモデル系におけるコスト／時間／スループット