Perspective

Clinical Cancer Research

Personalized Nanomedicine

Twan Lammers[1,2,3], Larissa Y. Rizzo[1], Gert Storm[2,3], and Fabian Kiessling[1]

Abstract

Personalized medicine aims to individualize chemotherapeutic interventions on the basis of *ex vivo* and *in vivo* information on patient- and disease-specific characteristics. By noninvasively visualizing how well image-guided nanomedicines—that is, submicrometer-sized drug delivery systems containing both drugs and imaging agents within a single formulation, and designed to more specifically deliver drug molecules to pathologic sites—accumulate at the target site, patients likely to respond to nanomedicine-based therapeutic interventions may be preselected. In addition, by longitudinally monitoring how well patients respond to nanomedicine-based therapeutic interventions, drug doses and treatment protocols can be individualized and optimized during follow-up. Furthermore, noninvasive imaging information on the accumulation of nanomedicine formulations in potentially endangered healthy tissues may be used to exclude patients from further treatment. Consequently, combining noninvasive imaging with tumor-targeted drug delivery seems to hold significant potential for personalizing nanomedicine-based chemotherapeutic interventions, to achieve delivery of the right drug to the right location in the right patient at the right time. *Clin Cancer Res; 18(18); 4889–94. ©2012 AACR.*

Introduction

Personalized medicine is often heralded as one of the major leaps forward for 21st century medical practice (1). It aims to individualize therapeutic interventions, incorporating not only information obtained using *ex vivo* genetic and proteomic profiling, but also *in vivo* imaging insights on the type, the stage, and the grade of the disease, as well as on the response of a particular patient to a particular treatment.

Ex vivo profiling

By quantifying the expression levels of certain genes and proteins in healthy versus pathologic tissues, *ex vivo* biomarker profiling primarily serves to predict how well a given patient might respond to a given therapeutic intervention, and how likely he or she is to develop side effects (2). Genotyping patients has, for instance, been shown to be highly useful for assuring optimal efficacy and minimal toxicity in case of treatment with the anticoagulant warfarin (CYP2C9 and VKORC1 polymorphisms; ref. 3), and with thiopurine- and irinotecan-based chemotherapeutics (TPMT and UGT1A1 polymorphisms, respectively; ref. 4). Similarly, immunohistochemical tests evaluating the protein expression levels of HER2, epidermal growth factor receptor (EGFR), and c-kit in metastatic breast, colorectal, and gastrointestinal tumors, respectively, are approved by the U.S. Food and Drug Administration (FDA) for enabling (more) personalized treatment with trastuzumab (Herceptin; Genentech), cetuximab (Erbitux; ImClone/Eli Lilly), and imatinib [Gleevec; Novartis (ref. 1)]. In recent years, such single-gene screening strategies have expanded toward whole-genome profiling and clustering analyses, which provide significantly larger amounts of diagnostic and prognostic information, and which likely are much more suitable for substantiating the choice for a particular (chemotherapeutic) treatment, for adapting its dosing regimen, and for predicting its outcome. As an example, genetic profiling of low- and medium-grade gliomas has recently shown striking differences between proneural, mesenchymal, and proliferative/classical phenotypes, which have markedly different clinical courses, and therefore, likely require completely different treatments and treatment regimens to assure high efficacy and low toxicity (5). Incorporating such high-throughput profiling and clustering analyses, and identifying genetic signatures rather than deregulated single genes, therefore seems to be an important way forward for improving and individualizing chemotherapeutic interventions. It should be taken into account in this regard, however, that both *ex vivo* single biomarker analyses and *ex vivo* genetic signature profiling primarily aim to *a priori* subdivide relatively large cohorts of patients into those likely to respond to a particular treatment, those unlikely to respond, and/or those likely to develop side effects. Consequently, the term "stratified medicine," rather than personalized medicine, seems to be more appropriate

Authors' Affiliations: [1]Department of Experimental Molecular Imaging, RWTH Aachen University, Aachen, Germany; [2]Department of Pharmaceutics, Utrecht University, Utrecht; and [3]Department of Targeted Therapeutics, University of Twente, Enschede, The Netherlands

Corresponding Author: Twan Lammers, Department of Experimental Molecular Imaging, RWTH Aachen University Clinic, Pauwelsstrasse 30, 52074 Aachen, Germany. Phone: 49-241-8036681; Fax: 49-241-803380116; E-mail: tlammers@ukaachen.de

doi: 10.1158/1078-0432.CCR-12-1414

Translational Relevance

Nanomedicine-mediated drug targeting, together with noninvasive imaging information on the biodistribution, the target site accumulation, and the therapeutic efficacy of drug delivery systems, might hold significant translational potential for improving the balance between the efficacy and the toxicity of systemic drug treatment. In the present article, the authors describe how combining diagnostic and therapeutic properties within a single nanomedicine formulation can be used to preselect patients in (early-phase) clinical trials, to adapt treatment regimens on the basis of biodistribution and efficacy monitoring, and to thereby individualize tumor-targeted nanochemotherapeutic interventions.

for such gene- and/or protein-based semi-individualized treatment regimens (6).

In vivo imaging

The latter of the abovementioned paths to personalized medicine (i.e., the implementation of noninvasive *in vivo* imaging) has not yet been extensively evaluated to date. Diagnostic information obtained with such modalities as positron emission tomography (PET), MRI, computed tomography (CT), and ultrasound is routinely used to assess the type, the stage, and the severity of a given pathologic condition (7); these insights, however, have thus far merely been used to assign relatively large cohorts of patients to a given therapeutic intervention (cf. stratified medicine; ref. 6), and not to really personalize the choice for a particular drug, its dose, and/or its dosing regimen. This is primarily because no suitable contrast agents are available for sensitively and specifically visualizing gene and protein expression by target cells and tissues. In the case of HER2-overexpressing breast carcinomas, for instance, it has been shown that radiolabeled trastuzumab is relatively useful for detecting novel and previously unidentified tumor lesions (in 13 of 15 patients), but not for detecting tumors in general (only 45% of previously identified HER2-positive tumors could be visualized; ref. 8), indicating that its physicochemical and its pharmacokinetic properties are suboptimal for proper disease diagnosis, and therefore also for preselecting patients and for enabling personalized medicine.

Follow-up experiments confirmed this observation, showing that because of its size and its prolonged circulation time (the blood half-life of trastuzumab is in the order of days), it is not very suitable for molecular imaging purposes, that is, for visualizing receptor expression with high specificity and high signal-to-noise ratios (9). These findings were corroborated by preclinical studies showing that an approximately 10-fold smaller trastuzumab-like affibody, which has a much shorter circulation half-life time, presented with much more favorable tumor-to-background ratios and was significantly more effective in specifically identifying HER2-positive tumors (10), as well as by studies also showing that the tumor accumulation of radiolabeled cetuximab was primarily dominated by its physicochemical and its pharmacokinetic properties, and not by receptor expression levels (11). Together, these efforts exemplify that noninvasively imaging the target site accumulation of radiolabeled antibodies does not hold much potential for facilitating personalized medicine, and that so-called companion diagnostics (12), with much shorter circulation times and much higher signal-to-noise ratios, are needed to individualize molecularly targeted chemotherapeutic interventions.

Nanotheranostics

This situation is very different for image-guided nanomedicines, nowadays routinely referred to as nanotheranostics. Nanotheranostics are submicrometer-sized carrier materials containing both drugs and imaging agents within a single formulation (13). As standard (non–image-duided) nanomedicines, they aim to improve the biodistribution and the target site accumulation of systemically administered chemotherapeutic drugs. By more effectively and more selectively delivering conjugated or entrapped drug molecules to the target site, and by at the same time preventing them from accumulating in potentially endangered healthy tissues, nanomedicine formulations aim to improve the balance between the efficacy and the toxicity of systemic chemotherapeutic interventions (14–16). For obvious reasons (e.g., high medical need, low efficacy, and toxicity of routinely used chemotherapeutic drugs), most efforts in the nanomedicine field have focused on cancer (17–19). As a result of these efforts, several chemotherapy-containing nanomedicine formulations have been approved for clinical use, including, for example, Myocet [non-PEGylated liposomal doxorubicin (Cephalon) for metastatic breast cancer], Doxil [PEGylated liposomal doxorubicin (Janssen); for metastatic breast cancer, ovarian cancer, multiple myeloma, and Kaposi sarcoma]; and Abraxane (albumin-based paclitaxel (Celgene); for metastatic breast cancer]. In most cases, however, these formulations are only moderately effective, and the primary reason for their approval and their routine clinical use has been their ability to reduce the toxicity of systemic chemotherapeutic treatments (19, 20).

This observation indicates that nanomedicine-based tumor-targeted interventions, like antibody-based molecularly targeted interventions, might profit substantially from noninvasive imaging insights on patient personalization. An important difference in this regard, however, relates to the fact that in the case of the former, the primary aim of incorporating noninvasive imaging techniques "merely" is to identify patients presenting with a sufficiently high level of target site accumulation, whereas in the case of the latter, it aims to preselect patients based on receptor expression. Visualizing and quantifying the overall level of target site accumulation is much less complicated than specifically

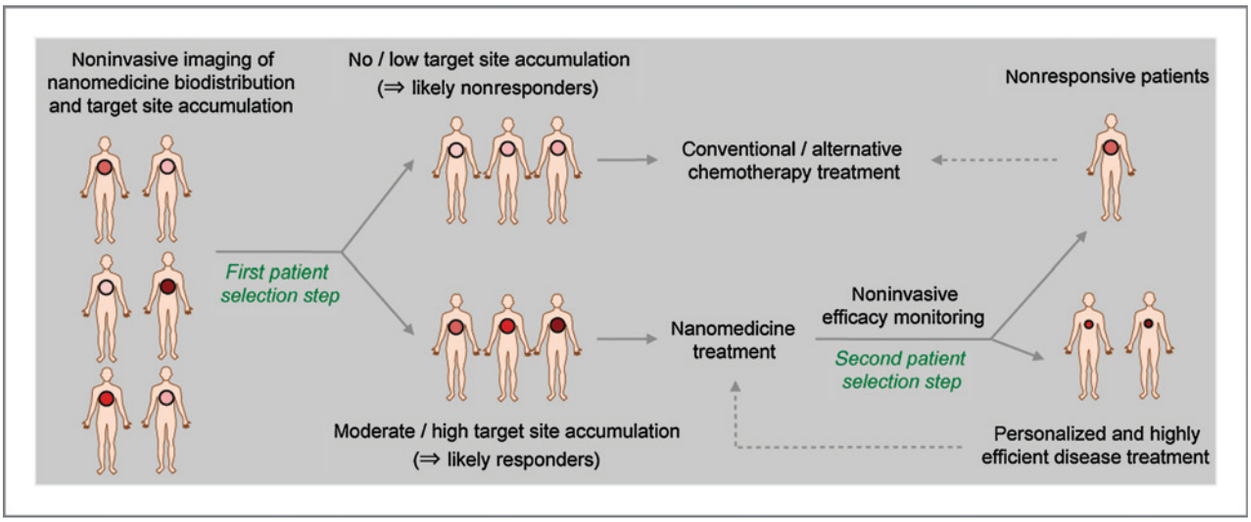

Figure 1. Rationale for personalized nanomedicine. By incorporating noninvasive imaging information on the target site accumulation (first patient selection step) and the therapeutic efficacy (second patient selection step) of nanomedicine formulations, patients can either be assigned to nanomedicine treatment (in case of moderate to high tumor accumulation and proper antitumor efficacy) or to conventional or alternative chemotherapeutic interventions (in case of low tumor accumulation and/or improper efficacy). In addition, during the first selection step, patients presenting with high levels of nanomedicine accumulation in potentially endangered healthy organs or tissues can be excluded from nanomedicine treatment to attenuate the incidence and/or intensity of side effects.

visualizing receptor expression by target cells. Therefore, preselecting patients based on noninvasive *in vivo* imaging is much more straightforward in tumor-targeted therapeutics than in molecularly targeted therapeutics. Strikingly, however, as opposed to a significant number of (semi-) unsuccessful studies on the potential of using radiolabeled antibodies to individualize systemic chemotherapeutic interventions (8–11), no systematic studies have been conducted thus far aiming to use noninvasive imaging information to personalize nanomedicine-based chemotherapeutic treatments.

Personalized nanomedicine

To bridge this translational gap, we here propose "personalized nanomedicine" as a novel, rational, and relatively straightforward concept for individualizing tumor-targeted chemotherapeutic interventions. As exemplified by Fig. 1, upon labeling nanomedicine formulations with contrast agents, the first step toward personalized nanomedicine treatment is to preselect patients on the basis of noninvasive imaging insights on target site accumulation. Then, patients presenting with moderate to high levels of target site accumulation are treated with the image-guided nanomedicine formulation in question, whereas those patients who do not are either allocated to conventional chemotherapy or to another experimental intervention. Subsequently, during the second personalization step, preselected and nanomedicine-treated patients are closely monitored during follow-up to noninvasively visualize how well they respond to the first 1 to 3 cycles of treatment. During this process, by means of continuous input from noninvasive imaging and reiteration, drug doses and dosing regimens can be adapted, and patients can be allocated to other (nano)-therapeutic interventions, if necessary.

Clinical case studies providing proof-of-principle for personalized nanomedicine treatment are depicted in Fig. 2. Figure 2A shows the biodistribution of a liver-targeted polymer–drug conjugate (i.e., PK2; galactosamine-modified pHPMA-GFLG-doxorubicin; targeted to the asialoglycoprotein receptor, which is overexpressed by hepatocytes), convincingly showing efficient target site localization (21). More detailed molecular imaging of the target site accumulation of PK2 using single photon emission CT (SPECT; Fig. 2B), however, coupled to anatomical CT imaging of the hepatocellular carcinoma (HCC) in question (Fig. 2C), showed that this targeted nanomedicine formulation primarily localized to healthy liver tissue, and not to the tumor. This observation likely explains why PK2 was found to be relatively ineffective for treating HCC, with clear-cut responses only observable in 3 of 31 patients (21).

Conversely, as shown in Fig. 2D, radiolabeled PEGylated liposomes accumulated highly efficiently in the primary tumor mass (in the lower left leg) of a patient with Kaposi sarcoma, as well as in a number of secondary and/or metastatic lesions (e.g., in the right shoulder and facial region; ref. 22). This observation explains, at least to some extent, why patients suffering from Kaposi sarcoma, which is characterized by a dense, highly leaky, enhanced permeability and retention (EPR; ref. 23)–prone vascular network, generally respond well to Doxil treatment. In a large phase III trial, for instance, in which Doxil was compared with the combination of doxorubicin (Adriamyxin), bleomycin, and vincristine (ABV), it produced 1 complete response and 60 partial responses, as compared with "only" 31 partial responses for ABV (24). It should be noted, however, that none of the above studies undertook efforts to correlate target site accumulation with therapeutic efficacy. Therefore, although it seems obvious that the higher the degree of

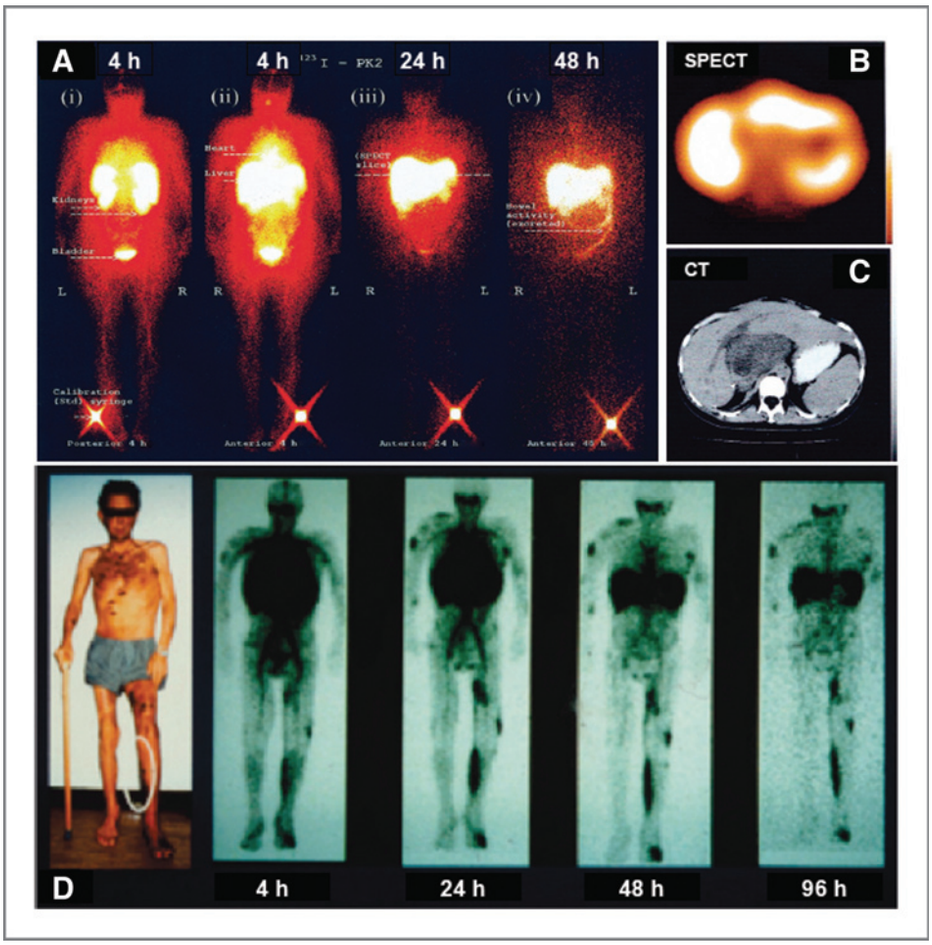

Figure 2. Clinical examples providing proof-of-principle for image-guided and personalized nanomedicine treatment. A, noninvasive gamma camera imaging of drug targeting to the liver using an iodine-123–labeled galactosamine-modified polymer–drug conjugate (i.e., Gal-pHPMA-GFLG-doxorubicin; PK2). B and C, molecular SPECT imaging of the accumulation of PK2 in the liver (B), coupled with anatomic CT imaging of the liver tumor (C), exemplifying the inefficient localization of PK2 to the dark HCC mass, and explaining why PK2 was found to be ineffective for treating HCC. D, efficient localization of indium-111–labeled PEGylated liposomes in primary (lower left leg) and secondary (upper left leg, right shoulder, and facial region) tumor lesions in a patient with Kaposi sarcoma (KS), explaining, at least in part, why Doxil is relatively effective for treating KS. Images adapted, with permission, from (21, 22).

target site localization of a given nanomedicine formulation is, the more effective it will be, no definite conclusions can be drawn from these studies yet. Consequently, we strongly urge scientists and clinicians working on tumor-targeted nanomedicines to incorporate noninvasive imaging in their trials, and to ideally not only do so during initial biodistribution and target site accumulation experiments, but also during follow-up, to monitor how well individual patients respond to nanomedicine treatment, and to correlate treatment efficacy with target site accumulation.

In addition, scientists, clinicians, and pharmaceutical companies working on tumor-targeted nanomedicines should realize that image guidance might be highly useful during the initial phases of clinical testing. In such early-phase trials, it would be very helpful to know which patients present with tumors characterized by high EPR (and/or by a low interstitial fluid pressure; refs. 19, 23), and which consequently show a high level of target site accumulation. If EPR turns out to be absent for a particular nanomedicine formulation for example, in 60% of patients, then it is likely that these 60% will not respond well to this formulation. Conversely, the 40% of patients presenting with relatively prominent EPR will likely also respond relatively well to treatment with this formulation. Consequently, incorporating noninvasive imaging insights on the target site accumulation of theranostic nanomedicines is considered to be highly useful for preselecting patients in early-phase clinical trails, and therefore likely also for facilitating and fostering the translation of tumor-targeted nanomedicines into routine clinical practice.

Clinical translation

To successfully translate personalized nanomedicine into the clinic, a number of hurdles need to be overcome:

1. The formulations that are already used in patients, as well as those that are close to clinical translation, should enable labeling with contrast agents. Given their physicochemical versatility, however, and the large amount of preclinical studies already undertaken to visualize the biodistribution and the target site accumulation of nanomedicine formulations in animal models, it is likely that labeling the vast majority of clinically relevant nanomedicines with, for example, radionuclides, will not be too problematic.
2. In-depth knowledge needs to be obtained on the (non-linear) pharmacokinetics of nanomedicine formulations. For drug-free radiolabeled liposomes, for

instance, it is known that they are excreted relatively rapidly at low lipid doses and upon repeated administration, by means of the so-called accelerated blood clearance (ABC) phenomenon (25). For repeated administration, it has also been shown that the presence of a chemotherapeutic drug abolishes ABC, thereby underlining the importance of including both drugs and imaging agents within a single theranostic nanomedicine formulation when intending to obtain really meaningful information on its pharmacokinetics and its target site accumulation (26).

3. As already alluded to above, it will be crucial to show that the degree of tumor accumulation of a given nanomedicine formulation corresponds, at least to some extent, with its therapeutic efficacy. Though it is highly likely that patients who present with high levels of target site accumulation will also respond well to tumor-targeted chemotherapeutic interventions, no systematic studies have been reported in this regard thus far, neither preclinically nor clinically. As expected, however, initial observations in animals do seem to confirm that improved target site localization (27) and increased drug release at the target site (28) correlate with improved therapeutic efficacy. Therefore, we again underline the importance of including noninvasive imaging information on nanomedicine biodistribution and tumor accumulation in late-stage preclinical and early-stage clinical trials.
4. Assuming that there will be a clear correlation between tumor concentration and therapeutic efficacy, it will be highly important to properly differentiate between low and high levels of target site accumulation. At present, it remains elusive from what (relative) percentages of the injected dose onwards, patients can be considered to present with (sufficiently) high levels of target site accumulation. Consequently, as it is likely that these values will vary from formulation to formulation, and from type of malignancy to type of malignancy, systematic and well-constructed clinical studies are needed to provide answers to such questions.
5. Information should be obtained on how the level of target site accumulation of nanomedicine formulations changes during the course of therapy. If, for instance, the size, stage, perfusion, and/or permeability of tumors decreases significantly during the first 2 to 3 cycles, and if this also substantially lowers the degree of nanomedicine accumulation, then it will imperative to establish parameters and protocols to decide whether or not to (dis-) continue nanomedicine treatment.
6. It will be highly important to investigate how targeting and treating primary tumors correlates with drug targeting to and drug treatment of metastases. Especially in end-stage patients—those generally confronted within early-phase clinical trials—long-term treatment efficacy will not be determined by the size of the primary tumor, but by metastatic burden. Therefore, noninvasive imaging information, both on the localization of metastases, and on the ability of nanomedicines to accumulate in and treat metastases, will be indispensable for furthering their clinical development.

In addition to providing noninvasive information on target site accumulation and/or on therapeutic efficacy, image-guided nanomedicines might also enable a more personalized prediction of potential off-target effects. If, for example, due to comorbidity or altered hepatic or renal excretion, a given nanomedicine formulation would unexpectedly accumulate very strongly in a potentially endangered healthy organ or tissue, such as the heart, the brain, or (part of) the gastrointestinal tract, then this would already become obvious during the initial patient selection step, and would lead to the exclusion of this particular patient from further nanomedicine treatment. As for imaging target site localization, however, it will be highly important in this regard to establish quantitative measures for differentiating high and low degrees of off-target accumulation, in order to predict from which percentages of the injected dose onward unacceptable side effects are likely to develop.

Furthermore, in the somewhat more distant future, it is expected that in addition to its use for personalizing tumor-targeted interventions via noninvasive imaging, nanomedicine—in its broadest sense—can also be implemented for the advanced detection of patient- and/or disease-specific biomarkers. Analogous to the *ex vivo* genetic and proteomic profiling efforts mentioned above, nanomedicine-based sensors for single-molecule detection (29, 30), and nanotechnology-optimized lab-on-a-chip systems (31) might, one day, complement *in vivo* imaging-guided nanotheranostic interventions, thereby providing an even more solid basis for personalized nanochemotherapeutic treatments.

Conclusions

Together, these insights indicate that noninvasive *in vivo* imaging can contribute substantially to realizing the potential of tumor-targeted and personalized nanomedicine, not only by preselecting patients in early-phase clinical trials, but also by allowing for individualized and optimized chemotherapeutic interventions once a given nanomedicine formulation has been approved for clinical use. Consequently, incorporating both drugs and imaging agents within a single nanomedicine formulation, and using the information that can be obtained with theranostic formulations to predict how well individual patients will respond to a particular tumor-targeted intervention, seems to be one of the most important and one of the most promising paths toward personalized nanomedicine. Such setups would enable personalized medicine to expand from the current and broadly accepted genetically reductionist version, which has not really paid off thus far (32), to more multidisciplinary and more advanced approaches, in which imaging is combined with drug targeting (and potentially also with molecular medicine), to achieve delivery of the right drug to the right location in the right patient at the right time.

Disclosure of Potential Conflicts Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: T. Lammers, L. Y. Rizzo, G. Storm
Writing, review, and/or revision of the manuscript: T. Lammers, F. Kiessling

Grant Support

This work was supported by grants DFG (LA 2937/1-1), DAAD, Hightech. NRW (ForSaTum) and EC (COST-TD1004).

Received April 29, 2012; revised June 12, 2012; accepted June 29, 2012; published OnlineFirst July 24, 2012.

References

1. Hamburg MA, Collins FS. The path to personalized medicine. N Engl J Med 2010;363:301–4.
2. Van 't Veer LJ, Bernards R. Enabling personalized cancer medicine through analysis of gene-expression patterns. Nature 2008;452:564–70.
3. Rieder MJ, Reiner AP, Gage BF, Nickerson DA, Eby CS, McLeod HL, et al. Effect of VKORC1 haplotypes on transcriptional regulation and warfarin dose. N Engl J Med 2005;352:2285–93.
4. Lesko LJ, Woodcock J. Translation of pharmacogenomics and pharmacogenetics: a regulatory perspective. Nat Rev Drug Discov 2004;3:763–9.
5. De Groot JF, Sulman EP, Aldape KD. Multigene sets for clinical application in glioma. J Natl Compr Canc Netw 2011;9:449–56.
6. Trusheim MR, Berndt ER, Douglas FL. Stratified medicine: strategic and economic implications of combining drugs and clinical biomarkers. Nat Rev Drug Discov 2007;6:287–93.
7. Weissleder R, Pittet MJ. Imaging in the era of molecular oncology. Nature 2008;452:580–9.
8. Perik PJ, Lub-De Hooge MN, Gietema JA, van der Graaf WT, de Korte MA, Jonkman S, et al. Indium-111-labeled trastuzumab scintigraphy in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer. J Clin Oncol 2006;24:2276–86.
9. Dijkers EC, Oude Munnink TH, Kosterink JG, Brouwers AH, Jager PL, de Jong JR, et al. Biodistribution of 89Zr-trastuzumab and PET imaging of HER2-positive lesions in patients with metastatic breast cancer. Clin Pharmacol Ther 2010;87:586–92.
10. Orlova A, Wallberg H, Stone-Elander S, Tolmachev V. On the selection of a tracer for PET imaging of HER2-expressing tumors: direct comparison of a 124I-labeled affibody molecule and trastuzumab in a murine xenograft model. J Nucl Med 2009;50:417–25.
11. Aerts HJ, Dubois L, Perk L, Vermaelen P, van Dongen GA, Wouters BG, et al. Disparity between *in vivo* EGFR expression and 89Zr-labeled cetuximab uptake assessed with PET. J Nucl Med 2009;50:123–31.
12. Papadopoulos N, Kinzler KW, Vogelstein B. The role of companion diagnostics in the development and use of mutation-targeted cancer therapies. Nat Biotechnol 2006;24:985–95.
13. Lammers T, Kiessling F, Hennink WE, Storm G. Nanotheranostics and image-guided drug delivery: current concepts and future directions. Mol Pharmaceut 2010;7:1899–912.
14. Allen TM, Cullis PR. Drug delivery systems: entering the mainstream. Science 2004;303:1818–22.
15. Wagner V, Dullaart A, Bock AK, Zweck A. The emerging nanomedicine landscape. Nat Biotechnol 2006;24:1211–7.
16. Duncan R, Gaspar R. Nanomedicine(s) under the microscope. Mol Pharmaceut 2011;8:2101–41.
17. Cho K, Wang X, Nie S, Chen ZG, Shin DM. Therapeutic nanoparticles for drug delivery in cancer. Clin Cancer Res 2008;14:1310–6.
18. Davis ME, Chen ZG, Shin DM. Nanoparticle therapeutics: an emerging treatment modality for cancer. Nat Rev Drug Discov 2008;7:771–82.
19. Jain RK, Stylianopoulos T. Delivering nanomedicine to solid tumors. Nat Rev Clin Oncol 2010;7:653–64.
20. Lammers T, Kiessling F, Hennink WE, Storm G. Drug targeting to tumors: principles, pitfalls and (pre-) clinical progress. J Control Release 2012;161:175–87.
21. Seymour LW, Ferry DR, Anderson D, Hesslewood S, Julyan PJ, Poyner R, et al. Hepatic drug targeting: phase I evaluation of polymer-bound doxorubicin. J Clin Oncol 2002;20:1668–76.
22. Harrington KJ, Mohammadtaghi S, Uster PS, Glass D, Peters AM, Vile RG, et al. Effective targeting of solid tumors in patients with locally advanced cancers by radiolabeled pegylated liposomes. Clin Cancer Res 2001;7:243–54.
23. Maeda H, Wu J, Sawa T, Matsumura Y, Hori K. Tumour vascular permeability and the EPR effect in macromolecular therapeutics: a review. J Control Release 2000;65:271–84.
24. Northfelt DW, Dezube BJ, Thommes JA, Miller BJ, Fischl MA, Friedman-Kien A, et al. Pegylated-liposomal doxorubicin versus doxorubicin, bleomycin, and vincristine in the treatment of AIDS-related Kaposi's sarcoma: results of a randomized phase III clinical trial. J Clin Oncol 2005;16:2445–51.
25. Laverman P, Boerman OC, Oyen WJG, Corstens FHM, Storm G. *In vivo* applications of PEG liposomes: unexpected observations. Crit Rev Ther Drug Carrier Syst 2001;18:551–66.
26. Lammers T, Aime S, Hennink WE, Storm G, Kiessling F. Theranostic nanomedicine. Acc Chem Res 2011;44:1029–38.
27. Chauhan VP, Stylianopoulos T, Martin JD, Popovic Z, Chen O, Kamoun WS, et al. Normalization of tumour blood vessels improves the delivery of nanomedicines in a size-dependent manner. Nat Nanotechnol 2012;7:383–8.
28. Ponce AM, Viglianti BL, Yu D, Yarmolenko PS, Michelich CR, Woo J, et al. Magnetic resonance imaging of temperature-sensitive liposome release: drug dose painting and antitumor effects. J Natl Cancer Inst 2007;99:53–63.
29. Ferrari M. Cancer nanotechnology: opportunities and challenges. Nat Rev Cancer 2005;5:161–71.
30. Ferrari M, Philibert MA, Sanhai WR. Nanomedicine and society. Clin Pharmacol Ther 2009;85:466–7.
31. Dittrich PS, Manz A. Lab-on-a-chip: microfluidics in drug discovery. Nat Rev Drug Discov 2006;5:210–8.
32. Editorial. What happened to personalized medicine? Nat Biotechnol 2012;30:1.