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## Preclinical targeting of the tumor microenvironment

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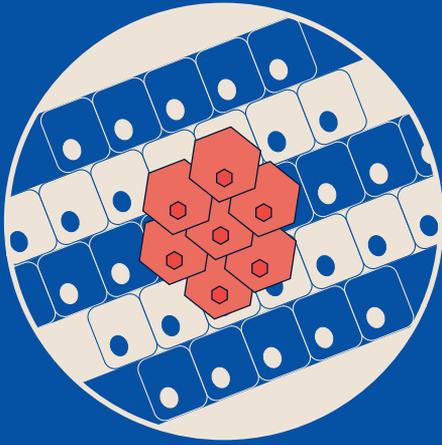
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# Chapter 6

## VEGF pathway targeting agents, vessel normalization and tumor drug uptake: from bench to bedside

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## **Abstract**

Vascular endothelial growth factor (VEGF) pathway targeting agents have been combined with other anticancer drugs, improving efficacy in a few cancer types. Vessel normalization induced by VEGF pathway targeting agents influences tumor drug uptake. Preclinical and clinical studies have shown a decrease in tumor delivery of radiolabeled antibodies and two chemotherapeutic drugs, following bevacizumab treatment. The decrease in vessel pore size during vessel normalization might explain the decrease in tumor drug uptake. Moreover the addition of bevacizumab to cetuximab or panitumumab in colorectal cancer patients or to trastuzumab in breast cancer patients, did not improve efficacy. However, combining bevacizumab with chemotherapy did increase efficacy in a few solid tumor types. Novel biomarkers to select patients who may benefit from combination therapies, such as the effect of an angiogenesis inhibitor on tumor perfusion, requires innovative trial designs and large clinical trials. Small imaging studies with radiolabeled drugs could be used in the interphase to gain further insight in the interplay between VEGF targeted therapy, vessel normalization and tumor drug delivery.

## Introduction

Angiogenesis, the formation of new blood vessels, is one of the hallmarks of cancer enabling tumor growth (1). Vascular endothelial growth factor A (VEGF-A) is one of the key players in the process of tumor angiogenesis, and the VEGF pathway has therefore been an important focus for anti-cancer drug development (2).

During antiangiogenic treatment the formation of new blood vessels is blocked. Initial high hopes of VEGF pathway targeting agents as panacea for treatment of solid tumors, have now been replaced by a more realistic definition of their role. Single agent activity has been shown in renal cell carcinoma, hepatocellular carcinoma, glioblastoma, pancreatic neuroendocrine tumors and ovarian cancers. VEGF pathway targeting drugs have been added to other anticancer drugs to obtain improved efficacy. However, this approach has only been successful in a few cancer types (3). Insight in the mechanisms involved may support rational combinations.

Preclinical and clinical studies indicate that anti-VEGF therapy induces changes in the function and architecture of existing blood vessels, described as vessel normalization.<sup>4</sup> Major characteristics of vessel normalization are reduced number and size of immature vessels, increased vessel pericyte coverage and reduced interstitial fluid pressure (4,5). The changes in the tumor induced by VEGF pathway directed drugs could also have an effect on tumor uptake of other drugs. It would be of great benefit when preclinical data could predict behavior of combination therapy in the clinic. At present, the translation of preclinical antiangiogenesis data to the clinic remains particularly challenging. Therefore this review focuses on the interplay between VEGF pathway targeting agents, vessel normalization and tumor drug delivery in the preclinical and clinical setting.

## Search strategies

Sources used to identify information for this paper are PubMed, ClinicalTrials.gov, NCBI, conference reports and references from relevant articles. The following search terms were used: “anti-angiogenic drugs”, “bevacizumab”, “VEGFR tyrosine kinase inhibitors”, “VEGFR2 antibody”, “ramucirumab”, “blood vessel normalization”, “tumor drug delivery/uptake”. Articles published in English between 2000 and 2014 were included.

## Vessel normalization and VEGF targeted agents

The vascular organization and structure in tumors differ from normal tissue. The tumor vasculature is more tortuous and chaotic, with inadequate pericyte coverage, increased breaches between endothelial cells and alternating thick and thin basement membranes. This leads to increased vessel permeability and high interstitial fluid pressure (IFP) causing hypoxia (4,5). Preclinical studies have shown that anti-VEGF therapy can initiate

the process of vessel normalization. Vessel normalization has been measured in the preclinical and clinical setting by a decrease in vessel diameter, blood volume, mean vessel density (MVD), macromolecular permeability, IFP and edema. Vessel normalization also leads to an increase in partial oxygen pressure and perivascular cell coverage in the tumor (4). In this review, vessel normalization is considered pruning and remodeling of abnormal tumor vessels, leading to vessels resembling the normal tissue vasculature in terms of structure and function (4,5).

To translate preclinical insights about vessel normalization to the clinic has been challenging. A reason for this remains the difference between experiments in tumor bearing mice and studies in patients. In general, murine models with subcutaneous, fast growing human tumors are being used (6). In patients, primary tumor lesions can be located anywhere in the body and are mainly slowly growing tumors, with doubling times of months to years compared to weeks in murine models. Even with metastatic disease, clinical progression is in general much slower than in the mouse model. In addition, xenograft models often comprise a single subcutaneously implanted human tumor lesion with murine vasculature. These tumors are treated for weeks, at most. In patients, of course both the tumor and vasculature are of human origin and long term treatment is required for optimal antitumor effect. In addition, the normal vasculature in patients will be aged, as for most cancers incidence rates increase with age (7). To improve translation from preclinical studies to the clinical setting, preclinical studies should ideally be representative for the stage of disease treated in the clinic, consist of tumor cells with a compatible immunocompetent microenvironment and examine combination therapies at appropriate dosages analogous to the clinic.

At the moment there are several agents targeting the VEGF pathway. Registered drugs targeting the VEGF pathway include small molecule tyrosine kinase inhibitors (TKI) targeting the VEGF receptors (VEGFR), and antibodies targeting VEGF and VEGFR2.

### **VEGFR TKIs**

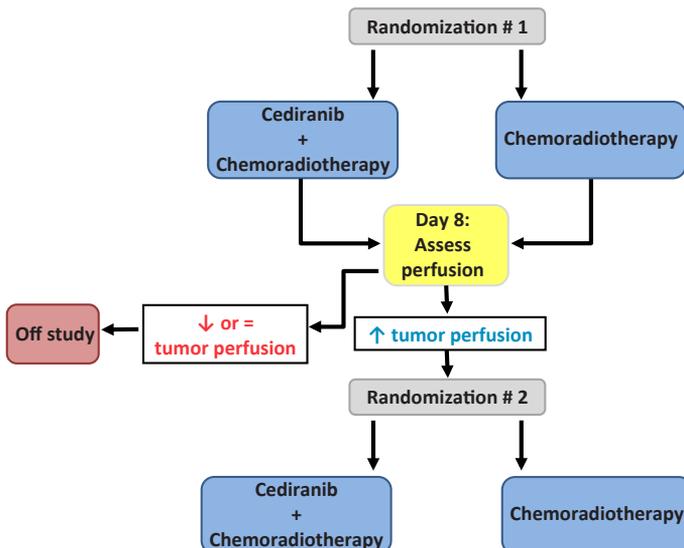
A recent meta-analysis evaluated the efficacy and safety of combining VEGFR TKIs with chemotherapy in solid tumor cancer patients.<sup>8</sup> Data of 24 randomized controlled trials with a total of 8,961 patients was included, with 879 patients participating in axitinib trials, 3,761 in sorafenib trials, 1,970 in sunitinib trials and 2,351 in vandetanib trials. The addition of VEGFR TKIs to chemotherapy increased side effects. There was an increase in any adverse events (relative risk 1.34, 95% confidence interval (CI) 1.20 - 1.50,  $P < 0.001$ ) and fatal adverse events (relative risk 1.49, 95% CI 1.16 - 1.90,  $P = 0.002$ ) (8).

Results from numerous phase 3 trials combining VEGFR TKIs with chemotherapy showed only marginal to no increased antitumor efficacy (6). In metastatic colorectal cancer, both

vatalanib in first- and second-line and sunitinib in first-line did not increase progression free survival (PFS) or overall survival (OS) when combined with chemotherapy (9-11). In the randomized phase 3 HORIZON II trial, the combination of cediranib with chemotherapy led to a clinically irrelevant increase of 0.3 months in PFS (HR 0.84,  $P = 0.012$ ), and had no effect on OS as first-line therapy in metastatic colorectal cancer patients (12). In addition, in metastatic breast cancer, sunitinib had no effect on PFS or OS when combined with chemotherapy as first- as well as second-line therapy (13,14). In non-small cell lung cancer (NSCLC), the addition of sorafenib to chemotherapy in the first-line had no effect on OS (15,16). Combining vandetanib with chemotherapy as second line NSCLC therapy in the randomized phase 3 ZODIAC trial ( $n=1,391$ ) led to an increase in PFS of 0.8 months (HR 0.79,  $P = 0.024$ ) (17). The smaller randomized phase 3 ZEAL trial ( $n=534$ ) trial did show a trend, but no significant increase in PFS when vandetanib was combined with chemotherapy in the second-line (18).

In recurrent glioblastoma multiforme (GBM) patients, combining cediranib with chemotherapy in the randomized phase 3 REGAL trial ( $n=325$ ) did not increase PFS and had no effect on OS (19). Of interest with regard to the vascular normalizing effects of cediranib, a small prospective study measured tumor blood perfusion changes with magnetic resonance imaging (MRI) during cediranib treatment in 30 recurrent GBM patients (20). Tumor perfusion increased in seven patients, decreased in 11 patients and remained stable in 12 patients. OS was prolonged to 348 days in patients with increased perfusion, compared to 169 or 213 days in patients with respectively stable or decreased perfusion ( $P = 0.019$ ). In another prospective phase 2 trial, patients with newly diagnosed GBM were allocated to receive 30 mg/day cediranib with chemoradiation ( $n=40$ ) or chemoradiation alone ( $n=14$ ) (21). The addition of cediranib led to an increase in perfusion in 20 (50%) patients, decreased perfusion in 10 (25%) patients and stable perfusion in 10 (25%) patients. These changes already occurred at day 1 and became stable from around day 8. However, also in one out of the 14 (7%) control patients, perfusion increased with chemoradiation alone. In the combination group, increased perfusion was associated with a median OS of 26.3 months compared to 17.0 months for patients without increased perfusion ( $P = 0.028$ ). Based on MRI analyses, these two studies show that cediranib increased perfusion in a subgroup of GBM patients and that this increase correlated with increased survival. From these results it was suggested, that increased tumor perfusion by cediranib induced vessel normalization might lead to increased tumor drug uptake and improve outcome in these patients as a result (20,21). Perfusion was suggested to be a potential read-out for vessel normalization and a possible predictive biomarker for survival in these patients. To provide insight in the implications of patient selection based on perfusion, we designed a hypothetical trial using an enrichment design for

primary GBM patients if these patients were to be treated by cediranib and/or standard chemoradiotherapy (Fig. 1) (22,23). In such a design, the biomarker is evaluated in all randomized patients, but only patients who are defined as biomarker-positive, i.e. patients with increased tumor perfusion after 8 days on initial randomized treatment, are eligible for a second randomization (23). To identify the biomarker-positive cohort, initially all patients are randomized to chemoradiotherapy with or without cediranib. In a second phase, only patients with increased perfusion after 8 days on treatment are randomized to continue chemoradiotherapy either with or without cediranib. The OS would be compared between randomized arms, to evaluate whether the addition of cediranib provides benefit in patients who achieved increased perfusion, irrespective of the initial treatment that led to this increase. Patients with decreased or stable perfusion would be taken off study and complete standard chemoradiotherapy. The second study phase, requires 310 patients, to achieve 80% power at a two-sided  $\alpha$  of 5%, assuming an OS improvement of 35% (HR = 0.65). This implicates that upfront 1,602 patients should have been entered for the first randomization, with 460 and 1142 patient initially randomized to treatment with or without cediranib (respectively corresponding to 230 and 80 patients with increased perfusion at 8 days under initial treatment). This is a considerably high number of GBM patients taking into account that the landmark paper demonstrating the additive effect of chemotherapy to first line radiotherapy in GBM required 573 patients, accrued during 20 months in 85 centers in 20 countries (22).



**Fig. 1** Scheme illustrating the randomization process for a hypothetical trial to evaluate tumor perfusion upon cediranib and chemoradiotherapy, as a predictive early biomarker for cediranib effect on survival in GBM patients.

## Bevacizumab

So far, combination studies of another antibody with bevacizumab plus chemotherapy have shown a detrimental to only a modestly beneficial effect. For metastatic colorectal cancer, combining bevacizumab, the anti-EGFR antibody cetuximab and chemotherapy in the phase 3 CAIRO2 trial did not improve OS compared to bevacizumab with chemotherapy alone. In fact, the addition of cetuximab even decreased PFS by 1.2 months (HR 1.22,  $P = 0.01$ ) (24). In addition, patients receiving cetuximab, bevacizumab and chemotherapy experienced more cetuximab-related side-effects. Similar results were obtained in the phase 3 PACCE trial in metastatic colorectal cancer patients receiving bevacizumab and chemotherapy or bevacizumab, chemotherapy and the anti-EGFR antibody panitumumab. In the panitumumab group PFS decreased by 1.4 months (HR 1.27) and no effect was observed on OS (25).

In the phase 3 AVEREL trial in metastatic HER2 positive breast cancer patients chemotherapy with trastuzumab or a combination of chemotherapy, trastuzumab and bevacizumab was administered (26). No significant impact of bevacizumab was observed on PFS (13.7 months without bevacizumab versus 16.5 months with bevacizumab; HR 0.82,  $P = 0.07$ ). Furthermore, another randomized phase 3 trial in metastatic HER2 positive breast cancer patients also showed that the addition of bevacizumab to trastuzumab and chemotherapy did not increase PFS (11.1 months without bevacizumab versus 12.2 months with bevacizumab; HR 0.65,  $P = 0.10$ ) (27).

It has been suggested, that vessel normalization induced by antiangiogenic drugs could improve tumor delivery of other drugs when combined. In three small rectal cancer studies, comprising 5, 6 and 32 patients, vessel normalization induced by 5-10 mg/kg bevacizumab was investigated with biopsies, IFP measurements and functional computed tomography (CT) scans. At 12 days after bevacizumab administration, IFP had decreased and the fraction of vessels covered with pericytes had increased, while the permeability-surface area product remained stable. These findings indicate that bevacizumab did induce vessel normalization in these patients (28-30). However, this does not prove a direct relation of vessel normalization and improved tumor uptake of other drugs. Imaging with radiolabeled drugs potentially provides a tool to quantify tumor uptake of labeled drugs. This would allow direct evaluation of the effects of vessel normalization on tumor drug uptake. Both preclinical and clinical studies evaluated the effects of antiangiogenic therapy on tumor drug uptake (Table 1).

In the University Medical Center Groningen (UMCG), we have developed  $^{89}\text{Zr}$ -labeled bevacizumab and  $^{89}\text{Zr}$ -trastuzumab as tracers for positron emission tomographic (PET) scanning to visualize and quantify bevacizumab and trastuzumab biodistribution for both preclinical and clinical purposes (31-33). These tracers can provide insight in

how bevacizumab affects uptake of other antibodies. Despite the limitations of animal models, the human grade tracers do allow mirroring of the findings in the preclinical setting to the clinic. In mouse xenograft models of human ovarian and esophageal HER2 positive cancer (SKOV-3 and OE19), we evaluated tumor uptake of radiolabeled anti-HER2 antibody trastuzumab (SKOV-3 and OE19), IgG (SKOV-3) and bevacizumab (SKOV-3) before and after bevacizumab treatment with PET imaging (34). On day 6, after three doses of 5 mg/kg bevacizumab, tumor uptake decreased with 41% and 39% for trastuzumab in respectively SKOV-3 and OE19. For radiolabeled IgG and bevacizumab, tumor uptake decreased with respectively 28% and 44% in SKOV-3 after bevacizumab treatment. This indicates that bevacizumab treatment affected antibody tumor uptake negatively. In addition, bevacizumab reduced uptake of fluorescent labeled IgG compared to control. Bevacizumab therapy reduced MVD in the tumors and increased vessel pericyte coverage, illustrating both anti-vascular and vessel normalizing effects of bevacizumab therapy. Two other preclinical studies report similar findings of reduced tumor uptake of antibodies after anti-VEGF therapy (35,36). One 10 mg/kg dose of the cross-reactive anti-VEGF antibody B20-4.1 decreased tumor trastuzumab uptake by 50% after 2 days in a xenograft HER2+ breast cancer model (KPL-4) (35). Moreover, 10 mg/kg bevacizumab decreased tumor anti-EGFR antibody cetuximab uptake by 40% after 4 days in EGFR+ breast cancer xenograft models (SUM149 and SKBR3) (36). Importantly, these findings are also supported in the clinical setting, as a study in patients with renal cell carcinoma (n = 11) showed 47% decrease of <sup>89</sup>Zr-bevacizumab tumor uptake 2 weeks after one therapeutic infusion of 10 mg/kg bevacizumab (37). Thus, vessel normalization induced by bevacizumab seems to impair tumor delivery of antibodies.

In addition, two clinical imaging studies suggest that this lower uptake of other antibodies following bevacizumab is not limited to antibodies, but also affects tumor drug delivery of chemotherapeutic drugs. In NSCLC patients (n = 10) a single dose of 15 mg/kg bevacizumab reduced <sup>11</sup>C labeled-docetaxel tumor delivery with 22% after 5 hours and with 34% after 4 days (38). Moreover, tumor drug delivery of <sup>18</sup>F-5-fluorouracil decreased with 20.2% 24 hours after a single administration of 7.5 mg/kg bevacizumab in metastatic colorectal cancer patients (n = 5) (39). Phase 3 trials combining bevacizumab with chemotherapy show varying results. Bevacizumab combined with chemotherapy in colorectal cancer, ovarian cancer, cervical cancer and HER2-negative breast cancer has shown an increase in PFS (40-44).

## VEGFR2 antibodies

Intravital imaging showed that DC101, an antibody against mouse VEGFR2, induced vessel normalization in an orthotopic mammary tumor model (45). This particular study is a key paper as it did provide insight in the effect of vessel normalization on pore size of tumor vasculature. The nanoparticles used were quantum dots coated with polymeric imidazole ligand (PIL) ( $\phi = 12$  nm) or polyethylene glycol (PEG) ( $\phi = 60$  and  $120$  nm) (46). Pore size was determined by modeling the nanoparticle penetration rate, given as transvascular flux per unit vascular surface area. Vessel normalization by DC101 coincided with a decrease in pore size of tumor vasculature, resulting in an increase of the penetration rate of the small nanoparticles (12 nm) but no difference in penetration rate for the 60 and 120 nm size nanoparticles. This means that the effect of DC101 on the pore size was mainly based on the difference in the transvascular flux of the 12 nm particles in tumors with and without DC101 treatment. Moreover, in the E0771 xenograft model treated with DC101, for example, a large spread in transvascular flux of the 12 nm nanoparticles was already present in this group (from  $0.05 - 0.3 \mu\text{m s}^{-1}$ ). Such a significant variation results in a large uncertainty in the model outcomes on pore size, which was not discussed by the authors. Since antibodies have a size of approximately 12 nm, similar to the small nanoparticles, it was suggested that DC101-induced vessel normalization may also improve tumor drug delivery of antibodies (47). However, although their size is similar, there are substantial chemical differences between the nanoparticles used in the study and antibodies which may affect the penetration rate (45,46). First of all, their nanoparticles are spherically shaped, whereas antibodies are Y-shaped (48,49). Secondly, the mass(density) of the studied nanoparticles and antibodies may be significantly different. Furthermore, the chemistry of the outer shell of the nanoparticles used in this study is different to antibodies (45). The PIL coated 12 nm size nanoparticles mainly have methoxy ( $\text{R-O-CH}_3$ ) functional end groups in the outer shell, the PEG coated 60 and 125 nm particles have hydroxyl ( $\text{R-OH}$ ) functional end groups. The antibodies, which can be seen as a combination of four biopolymers, on the other hand, mainly have  $\text{R-NH}_2$  and  $\text{R-COOH}$  end groups (48,49). Because of these differences, it is not necessarily easy to translate the results regarding these specific nanoparticles to nanomedicines such as antibodies. Furthermore, this important study investigated whether DC101-induced vessel normalization could improve the efficacy of small chemotherapeutics (45). Mice were treated with DC101 or placebo, DC101 or placebo plus abraxane (albumin-bound paclitaxel,  $\phi = 10$  nm), DC101 or placebo plus doxil (liposome-encapsulated doxorubicin,  $\phi = 100$  nm). Tumor doubling times were used as a read-out for efficacy. Both, DC101 alone and DC101 plus doxil had no effect on tumor doubling times compared to placebo alone or doxil plus placebo. However, DC101 plus abraxane did increase tumor doubling

times, compared to abraxane plus placebo. From these data it was concluded that vessel normalizing effects of DC101 increased tumor penetration of abraxane. The results of the control experiments in this article showed a large range in tumor doubling times in the doxil plus placebo group. This might have influenced the results for this group.

In the clinic, the VEGFR2 antibody ramucirumab, administered at much lower doses than in the mouse model, has shown very modest or no effect in four phase III clinical trials. Ramucirumab monotherapy (8 mg/kg every 2 weeks) increased PFS by 0.8 months (HR: 0.48,  $P < 0.0001$ ) and prolonged OS by 1.4 months (HR: 0.77,  $P = 0.047$ ) in gastric cancer and esophageal junction adenocarcinoma patients in the second line (50). The FDA recently approved the drug for advanced gastric and esophageal junction adenocarcinoma patients. Adding ramucirumab (8 mg/kg every 2 weeks) to paclitaxel increased PFS from 2.9 months to 4.4 months (HR 0.63,  $P < 0.0001$ ) and prolonged OS by 2.3 months from 7.4 to 9.6 months (HR 0.80,  $P = 0.017$ ), in advanced gastric and esophageal junction adenocarcinoma patients (51). In addition, in NSCLC patients 10 mg/kg ramucirumab every 3 weeks combined with docetaxel increased PFS by 1.5 months (HR 0.76,  $P < 0.0001$ ) and prolonged OS by 1.4 months (HR 0.86,  $P = 0.023$ ) as second-line therapy (52). However, in breast cancer patients, 10 mg/kg ramucirumab every 3 weeks combined with docetaxel did not affect PFS or OS (53).

## Conclusion

To date, preclinical studies have shown that anti-angiogenic therapy can induce vessel normalization. Clinical studies have illustrated that this is not just a preclinical phenomenon, it also occurs in patients. Although some studies suggest that vessel normalization can improve drug delivery of chemotherapy and enhance efficacy of combination therapies, there is no direct evidence for better tumor drug uptake. On the contrary, both preclinical and clinical studies with radioactive labeled drugs have shown a decrease in tumor delivery of both antibodies and chemotherapy. In the case of chemotherapy, this did not inevitably result in absence of an additional effect of the combination therapy although it may explain disappointing results and lack of synergism. In the case of antibodies, results from clinical trials in colorectal and breast cancer patients are in line with reduced antibody uptake after antiangiogenic therapy. A possible explanation for a decrease in uptake, can be the change in vessel pore size during vessel normalization. This might influence tumor drug uptake, depending on size, shape and chemical construction of the drug. Most research concerning vessel normalization has been performed on primary tumors. Preclinical and clinical studies have shown that vessel normalization is a delicate process, occurring during a certain timeframe and dependent on the dose of the antiangiogenic drug (5). However, it remains unclear how

vessel normalization will occur in the metastasized setting. In normal healthy tissue, tissue-specific vessel functions illustrate vessel heterogeneity for different organs (54). This also applies to the tumor vasculature, and indeed, different types of tumor blood vessels have been identified (55). In NSCLC patients for instance, the primary tumor and their brain metastases differ in MVD, vessel maturity and VEGF expression (56). There is a need for a biomarker or tool to evaluate the process of vessel normalization in different tumor types and stages. A possible biomarker to select patients who may benefit from combinations of antiangiogenic and other drugs is tumor perfusion. However, to assess its role requires innovative study designs and extensive clinical trials. A possible tool to eventually improve drug delivery to individual tumors and thereby to optimize outcomes of combination therapies could be *in vivo* imaging of labeled drugs. This might clarify the interplay between vessel normalization and tumor drug delivery. Small clinical trials could be performed to visualize effects of antiangiogenic drugs on the distribution of other labeled drugs, provide serial information on whole body drug distribution, and guide rational trial design for large combinatorial studies.

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