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RAISEing VEGF-D's importance as predictive biomarker for ramucirumab in metastatic colorectal cancer patients

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A key unresolved question in the field of therapeutic antiangiogenesis is the lack of clinically applicable biomarkers to pre-select responding patients and spare resistant patients unnecessary side-effects and costs. The study by Tabernero et al. discovered VEGF-D as a novel biomarker candidate to predict efficacy of the anti-VEGFR-2 antibody ramucirumab as second-line treatment in metastatic colorectal cancer (mCRC) in the phase III RAISE trial [1].

This trial, which included only patients, who were refractory to bevacizumab, showed a survival benefit upon treatment with ramucirumab.FOLFIRI compared with the placebo.FOLFIRI group [2–6]. These results led to the approval of ramucirumab for the treatment of mCRC besides gastric, gastroesophageal junction and non-small-cell-lung cancer. Consistent with the results from most antiangiogenic therapy (AAT) trials, the survival benefit for the mCRC patients treated with ramucirumab was in the order of months. Unfortunately, until today, very few promising biomarker candidates have been identified, mostly from phase III trials with bevacizumab in different cancer types. These include short VEGF isoforms, modified expression of neuropilin-1, genetic variants and modified expression of VEGF receptor 1 (VEGFR1) [7–11]. However, there are no positive trials prospectively validating these candidates yet [12].

The investigators need to be congratulated for conducting a statistically sound prospective biomarker study attached to the trial. Notably, this included randomization of patients in an exploratory and confirmatory cohort, leading to well-balanced clinical characteristics between these subgroups. However, an outstanding question is why in the context of mandatory collection of plasma samples, ~20% of the patients were not included into the biomarker cohort [1, 2].

The translational biomarker studies within the RAISE trial included VEGF-C, VEGF-D, soluble (s) VEGFR-1, sVEGFR-2, and sVEGFR-3 in plasma samples and immunohistochemical evaluation of VEGFR-2 and vessel density [1, 2]. Of all biomarkers analyzed, only VEGF-D displayed predictive and prognostic significance. A cut-off value of 115 pg/mL VEGF-D was identified to discriminate between patients with low and high VEGF-D expression. High expression of VEGF-D was associated with beneficial progression-free survival (PFS) and overall survival (OS), but not objective response rate (ORR) upon addition of ramucirumab to chemotherapy [1] (Figure 1). In contrast, ramucirumab did not improve PFS nor OS in patients with low VEGF-D plasma levels when compared with the placebo.FOLFIRI arm.

VEGF-D binds to VEGFR-2 and VEGFR-3 and has multifaceted tumor-promoting effects including stimulation of tumor growth, angiogenesis, lymphangiogenesis and metastasis in addition to immunosuppression [13, 14]. It is thus not surprising that this growth factor of the VEGF family has a prognostic value besides its relevance as a predictive biomarker [1].

The potential of VEGF-D as predictive biomarker has already been retrospectively studied in two first-line CRC trials (CALGB 80405; AGITG MAX), both combining the anti-VEGF antibody bevacizumab with chemotherapy [15, 16]. In both trials, low VEGF-D levels were associated with better outcome upon treatment with bevacizumab. At first sight, these findings are apparently in contrast with the outcomes of the RAISE trial. However, although traditionally considered to mediate lymphangiogenesis, VEGF-D can substitute for the bevacizumab target VEGF-A and induce angiogenesis due to its binding capacity to VEGFR-2. Based on this biologic rationale, one can speculate that patients with high expression of VEGF-D would not benefit from bevacizumab but instead from ramucirumab (Figure 1). Along these lines, in the patient cohort enrolled in the RAISE trial, who progressed under therapy with bevacizumab, VEGF-D might be compensatorily upregulated and these patients seem to benefit most from the addition of ramucirumab to second-line treatment. Obviously, this assumption has several caveats, including different methodologies used to detect VEGF-D in the RAISE, CALGB and AGITG MAX trials [15, 16]. Surprisingly, the VEGFD levels detected in RAISE were about 10-fold lower than those of VEGF-D in CALGB [1]. If VEGF-D would mediate resistance to bevacizumab, one could expect higher levels of VEGF-D in the RAISE trial population, because in this second-line setting, all patients included were pre-treated with bevacizumab. These discrepancies in VEGF-D levels illustrate the necessity to develop a clinically applicable diagnostic enzyme-linked immunosorbent assay (ELISA). An interesting future trial design could

be to treat mCRC patients, in whom tumors are still progressing after EGFR inhibition, with bevacizumab or ramucirumab according to VEGF-D levels.

It is surprising that VEGF-C is neither predictive nor prognostic in RAISE, given the comparable affinity of VEGF-D and VEGF-C for VEGFR-2 [17, 18]. Also, both cytokines can induce angiogenesis via VEGFR-2 and VEGFR-3, and mouse data indicate that the combined genetic deletion of VEGF-C and VEGF-D does not have additional inhibitory effects on vascular growth compared with the deletion of VEGF-C alone [19]. Perhaps, VEGF-D has another (more important?) biologic role in pathologic angiogenesis mediating resistance to ramucirumab, which might distinguish it from VEGF-C—future work is needed to resolve this issue.

One point to consider is that the binding affinities of VEGF-C and VEGF-D for VEGFR-2 are increased by proteolytic cleavage, with only mature forms binding to VEGFR-2 [13, 20]. However, recent data indicate that distinct parts of the N-terminus (Phe₉₃–Arg₁₀₈) of cleaved VEGF-D are critical for binding to VEGFR-2, which is not the case for VEGF-C [21], indicating functional differences between these growth factors. At this point, it is not clear which of the VEGF-D variants is detected by the ELISA employed. Also, which cells produce VEGF-D in these mCRC patients? While VEGF-D mRNA is detectable in heart, lung and intestines [13, 22], possible candidates of VEGF-D-producing cells in mCRC patients can be cancer cells and tumor-infiltrating immune cells [23], though this remains to be confirmed in the future.

The prognostic and predictive relevance of VEGF-D raises the question of whether therapeutic targeting of VEGF-D could be an alternative option to blocking VEGFR-2. Monoclonal antibodies targeting VEGF-D were developed and might be useful as a single agent or in combination with bevacizumab to overcome resistance [21, 24]. Furthermore, combination strategies targeting both VEGFR-2 and VEGFR-3 might be considered, because they proved to be superior in comparison to VEGFR-3 or VEGFR-2 inhibition alone in mice, and inhibition of VEGFR-3 alone has shown to be well-tolerated but displays low efficacy in mCRC patients when given as monotherapy [24, 25]. In conclusion, the RAISE trial identified VEGF-D as a potential predictive biomarker, warranting independent validation.

Determination of VEGF-D levels could help to stratify patients for second-line mCRC therapy with ramucirumab and possibly for other indications. In addition to the identification of robust biomarkers for the efficacy of different AATs, alternative strategies, such as targeting endothelial cell metabolism to overcome resistance to current AATs, are warranted [14, 26].

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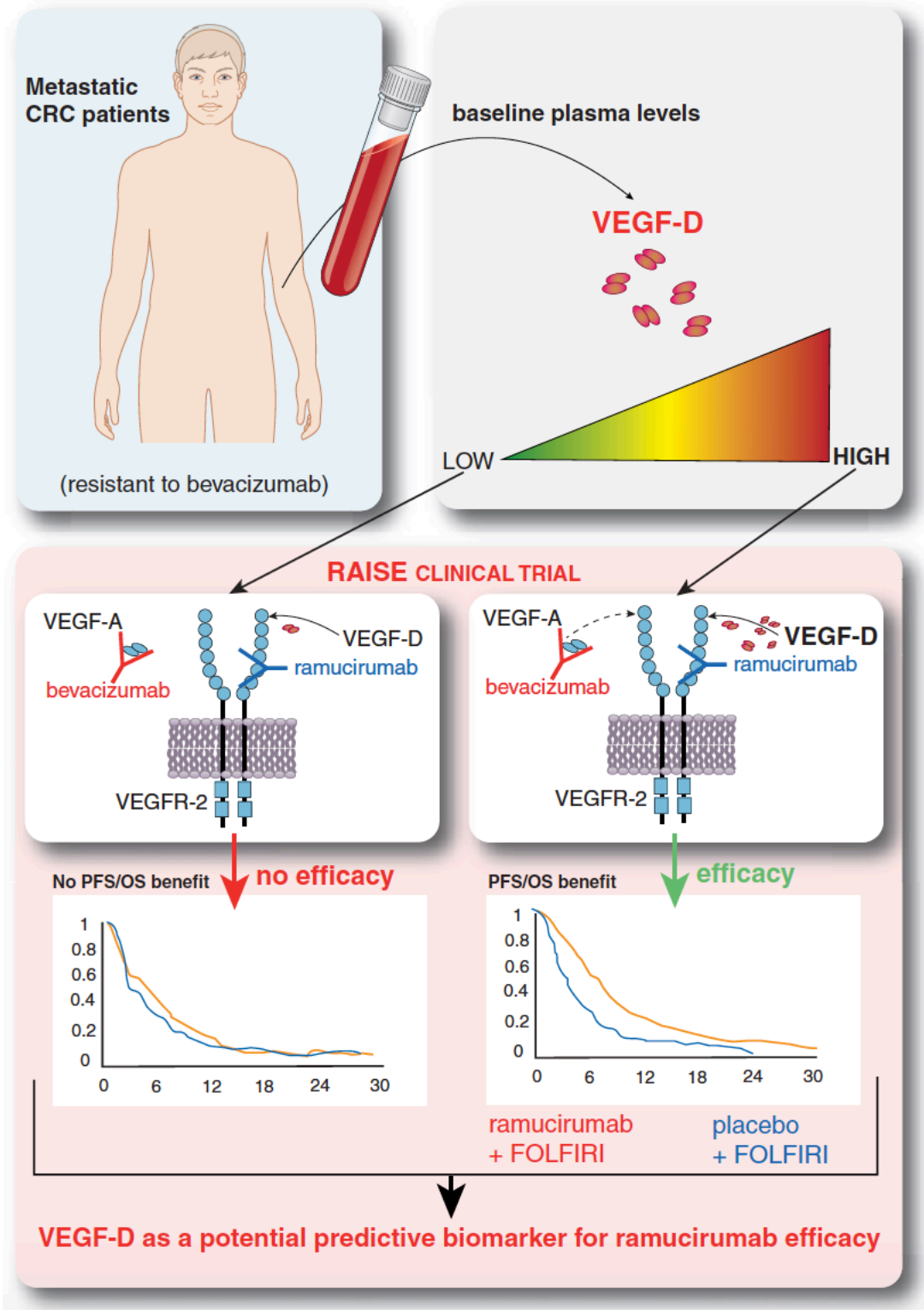


Figure 1. In RAISE, mCRC patients, resistant to bevacizumab, were sampled for possible biomarkers, of which only VEGF-D displayed predictive value for treatment with ramucirumab. The binding characteristics of the VEGF forms to VEGFR-2 are shown.