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Selecting Potential Targetable Biomarkers for Imaging Purposes in Colorectal Cancer Using TArget Selection Criteria (TASC): A Novel Target Identification Tool

Marleen van Oosten*, Lucia M.A. Crane*, Joost Bart†, Fijs W. van Leeuwen‡ and Gooitzen M. van Dam*

*Department of Surgery, Division of Surgical Oncology, Surgical Research Laboratory/BioOptical Imaging Center, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; †Department of Pathology and Medical Biology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; ‡Departments of Radiology and Nuclear Medicine, Division of Diagnostic Oncology, the Netherlands Cancer Institute, Amsterdam, The Netherlands

Abstract

Peritoneal carcinomatosis (PC) of colorectal origin is associated with a poor prognosis. However, cytoreductive surgery combined with hyperthermic intraperitoneal chemotherapy is available for a selected group of PC patients, which significantly increases overall survival rates up to 30%. As a consequence, there is substantial room for improvement. Tumor targeting is expected to improve the treatment efficacy of colorectal cancer (CRC) further through 1) more sensitive preoperative tumor detection, thus reducing overtreatment; 2) better intraoperative detection and surgical elimination of residual disease using tumor-specific intraoperative imaging; and 3) tumor-specific targeted therapeutics. This review focuses, in particular, on the development of tumor-targeted imaging agents. A large number of biomarkers are known to be upregulated in CRC. However, to date, no validated criteria have been described for the selection of the most promising biomarkers for tumor targeting. Such a scoring system might improve the selection of the correct biomarker for imaging purposes. In this review, we present the TArget Selection Criteria (TASC) scoring system for selection of potential biomarkers for tumor-targeted imaging. By applying TASC to biomarkers for CRC, we identified seven biomarkers (carcinoembryonic antigen, CXC chemokine receptor 4, epidermal growth factor receptor, epithelial cell adhesion molecule, matrix metalloproteinases, mucin 1, and vascular endothelial growth factor A) that seem most suitable for tumor-targeted imaging applications in colorectal cancer. Further cross-validation studies in CRC and other tumor types are necessary to establish its definitive value.

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Introduction

Patients with colorectal cancer (CRC) have an estimated 5-year survival, varying from approximately 90% in patients with stage I disease (Dukes A) to approximately 10% in patients with metastatic disease (Dukes D) [1]. Peritoneal carcinomatosis (PC) is a common form of end-stage colorectal cancer (CRC), affecting 10% to 15% of patients at the time of primary surgery and accounting for 25% to 35% of the recurrences of CRC [2]. PC has a median survival of 5 to 7 months without treatment [3–5].

Since the last decade, selected stage IV CRC patients with PC are treated with hyperthermic intraperitoneal chemotherapy (HIPEC). This procedure consists of flushing the intra-abdominal cavity with heated chemotherapy perioperatively after primary cytoreduction. HIPEC improves the median survival to 13 to 63 months, with a

Address all correspondence to: Gooitzen M. van Dam, MD, PhD, Department of Surgery, University Medical Center Groningen, Hanzeplein 1, 9700 RB Groningen, The Netherlands. E-mail: g.m.van.dam@chir.umcg.nl
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5-year survival varying from 19% to 51% [6–10]. However, further improvement is still desirable.

A more extensive surgical cytoreduction is associated with an increase in survival [11,12]. Furthermore, because penetration of chemotherapeutic drugs into peritoneally located tumor tissue is only superficial (limited to 1-2 mm), optimal cytoreduction by removing all visible tumor noduli is an essential prerequisite for the HIPEC procedure [13–15].

The limited survival in stage IV CRC asks for a more vigorous approach to improve prognosis. Current research is mainly focused on tumor-targeted imaging and therapy for diagnosis, treatment, and follow-up because these are expected to yield tumor-specific and thus stronger diagnostic and therapeutic effects. Therefore, objective identification of suitable tumor biomarkers for diagnostic and therapeutic purposes seems appropriate. Furthermore, tumor-targeted imaging can aid in identification of metastatic disease and in detection of recurrent disease. In this review, we emphasize tumor-targeted imaging because targeted therapeutics demand an entirely different approach for a meta-analysis.

A large number of biomarkers have been reported to play an important role in CRC. However, a limited number of these markers are suitable for tumor targeting based on characteristics such as, for example, expression rates. In literature, few objective data on how to determine the suitability of a potential target are available. Therefore, we set out to design a novel scoring system for classification and selection of biomarkers for tumor targeting applications. CRC is used as a clinical example for development and initial testing of this novel scoring system. With the emphasis on diagnostic and intraoperative imaging, we identified the most promising markers for tumor targeting in CRC using the scoring system.

In conclusion, in this review, we provide an overview of potential biomarkers for tumor targeting in CRC, supported by a newly designed TArget Selection Criteria (TASC) scoring system.

Methods

Design of the TASC Scoring System

Seven most important target characteristics selected based on the literature were summarized and granted 0 to 6 points, in order of importance. Subsequently, the selection system was tested by scoring a number of random biomarkers. Cutoff values were determined, and the scores were slightly adjusted where necessary to assure realistic outcomes. Finally, the selection system was further validated by testing a wide spectrum of biomarkers based on a publication of Cardoso et al. [16].

Literature Search Methods

Cardoso et al. [16] presented a table of genes found to be upregulated in CRC compared with normal colon tissue, as confirmed in three or more articles. The initial literature search query was based on this extensive table of genes. In addition, based on this table, we analyzed all genes mentioned for overexpression of the related protein because protein expression is not always synchronously upregulated, using Swiss-Prot and PubMed from 1985 to May 2010 (Figure 1). Furthermore, we included a number of proteins that were not mentioned in the table of Cardoso et al. but were otherwise described in the literature to play a significant role in CRC.

Finally, a systematic search of literature was performed, with PubMed as the main database, using the following search terms: the name of

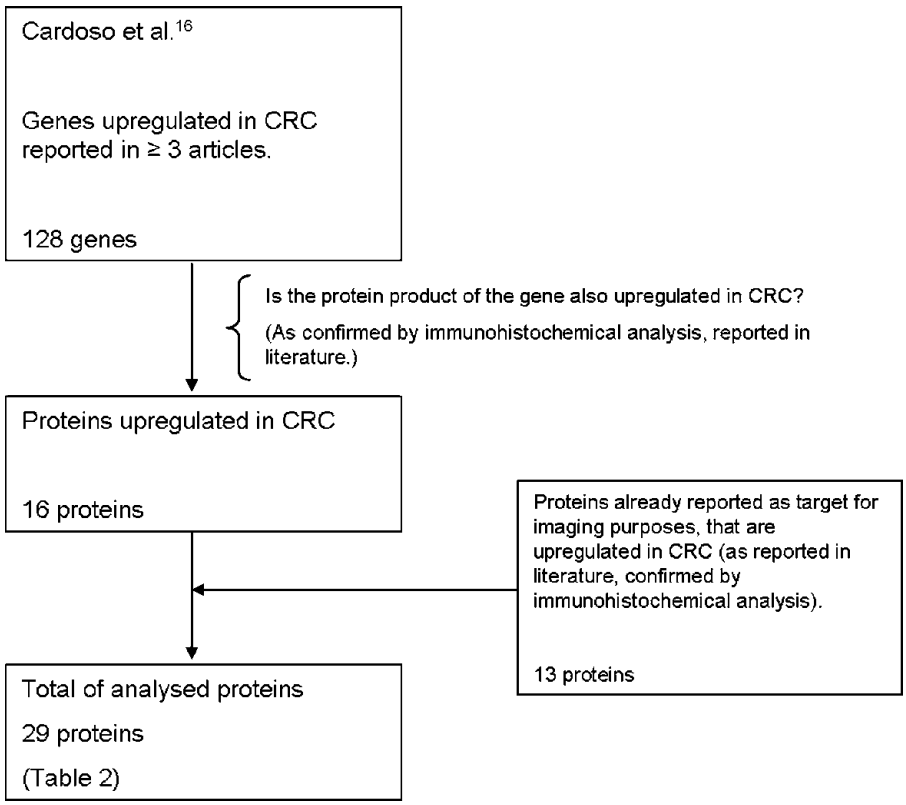

Figure 1. Selection of biomarkers upregulated in CRC.

the protein + "immunohistochemistry" + "colorectal cancer," and the name of the protein + "imaging" + "colorectal cancer," or variations of these terms, from 1985 to May 2010.

Target Selection: Introducing TASC

A tumor biomarker can be defined as a distinguishable component present on the tumor cell or secreted by a tumor cell to the surrounding stromal tissue. Such a biomarker is often a target in biologic interactions, e.g., the combination of CXC chemokine receptor 4 (CXCR4) as target of SDF-1. Alternatively, a biomarker can be used as a target for a synthetic substrate, which can be a single molecule, antibody, or others. Such a substrate can be conjugated to a diagnostic or imaging agent or a drug for clinical application purposes.

To our best knowledge, a scoring system to identify the most ideal target characteristics has never been explicitly described or developed or even validated. However, a number of favorable target features can be logically extracted from literature so far. On the basis of these characteristics, we propose a novel scoring system for target selection in particular for imaging purposes, the TASC.

The TASC score is based on the seven most favorable target characteristics that are granted points if it applies to the marker (Table 1). These characteristics are as follows: I) extracellular biomarker localization, either on the cell membrane or in close proximity of the tumor cell; II) expression pattern; III) tumor-to-healthy tissue ratio (T/N); IV) percentage of positive tumors; V) reported successful use of the biomarker in *in vivo* imaging studies; VI) enzymatic activity; and VII) internalization (Figure 2).

We will briefly explain these seven individual characteristics:

I—A target must be easily accessible by an agent, administered either systemically or intraperitoneally. For effective targeting, as little as possible barriers should be between the agent and its target. As a consequence, most conveniently, the marker is present on the cell surface. Alternatively, the expression of the target in the extracellular tumor matrix may also be adequate for imaging purposes. In our opinion, the extracellular localization of the target, either membrane-bound or near the tumor cell, is one of the most important factors and is therefore weighted substantially in the TASC system. Extra points are given to a cell membrane bound target because it is expected that membrane-bound targets more specifically emit signal from the tumor cell than soluble targets.

II—In the best scenario, the target is expressed by all tumor cells. However, in reality, this is very rare because cancer cells have the reputation of being heterogenic [17]. Also acceptable

Table 1. The TASC.

TASC Scoring System			
	Characteristics		Score
I	Extracellular protein localization	Bound to cell surface (receptor)	5
		In close proximity of tumor cell	3
II	Diffuse up-regulation through tumor tissue		4
III	T/N ratio > 10		3
IV	Percentage up-regulation in patients	>90%	6
		70%-90%	5
		50%-69%	3
		10%-49%	0
V	Previously imaged with success *in vivo*		2
VI	Enzymatic activity		1
VII	Internalization		1
Total: maximum 22			
Potential target ≥ 18			

A biomarker is granted points for seven factors (I-VII). A total score of 18 or higher indicates that the biomarker is potentially suitable for tumor-targeted imaging purposes.

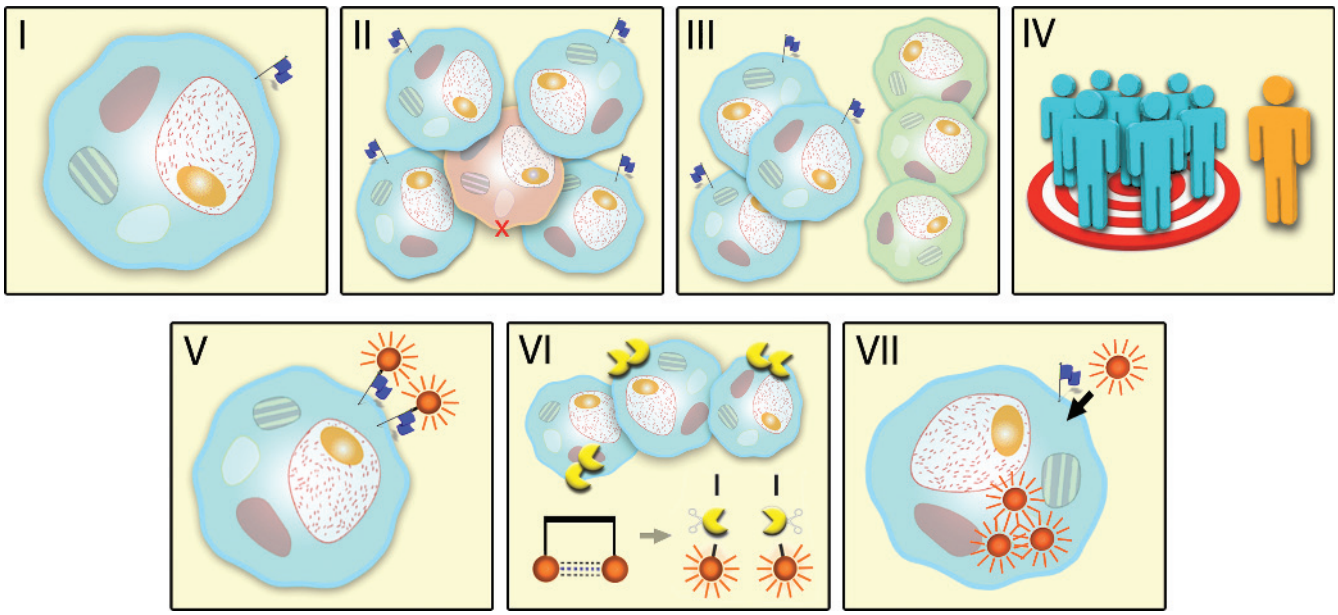

Figure 2. The TASC. The blue flag represents the selected biomarker. I. Extracellular localization of the biomarker, cell membrane–bound, or in close proximity of tumor cell. II. Diffuse up-regulation of the target throughout tumor tissue. III. T/N ratio greater than 10. Blue cells represent tumor cells; normal cells are green. IV. Up-regulation of the biomarker in most patients. V. A biomarker that has previously successfully been used in *in vivo* imaging studies. VI. Enzymatic activity facilitating the use of activatable probes. Shown are cleaving enzymes (yellow) that activate the imaging agent. VII. Internalization of probe for accumulation of imaging agent.

is a marker that is evenly distributed throughout the tumor tissue. High sensitivity to detect all tumor tissue is essential; therefore, this factor also has a significant power in TASC.

III—The expression of the biomarker should be minimal in normal tissue. In modalities like positron emission tomography (PET) and single photon emission computed tomography (SPECT) scanning, a tumor-to-healthy cell (T/N) ratio of greater than 10 is considered sufficient [18]. In fluorescence imaging, a minimal T/N ratio has not yet been described but is expected to be comparable to the previously mentioned modalities on the basis of its detection sensitivity and specificity.

IV—It is highly preferable that the use of a particular biomarker is of value for large patient populations rather than only small groups of "special" patients. Overexpression of the target in most patients increases clinical applicability of a tumor-targeted agent.

V—Previous use of a biomarker in *in vivo* imaging indicates suitability of the marker for imaging purposes in other diseases; in this case, CRC.

VI—Although not an absolute condition for a target, (extracellular) enzymatic activity in and around the tumor tissue offers the possibility of applying locally activated imaging agents, so-called *smart probes*, increasing the signal-to-background ratio [19].

VII—It is reported in the literature that internalization of the probe-target complex in the tumor can lead to intracellular accumulation of the imaging agent, which improves the signal and leads to a more optimal T/N ratio [20]. For this reason, internalization is granted points in the selection criteria.

Selecting a target that meets up to all of these conditions is challenging. In most cases, it is not necessary to meet all criteria.

A total score of 21 or 22 implies that a marker has a high potential for use as a target for imaging tracers *in vivo*. If a marker scores 18 or higher, it is considered to be a potential target. Markers with a score of less than 18 seem less suitable for targeted imaging modalities and require more research to evaluate their potential.

Possible Target Candidates in Colorectal Cancer

It is well known that it can be difficult to distinguish cancer cells from its normal surroundings because of the many similarities between malignant cells and normal cells. Furthermore, tumors are mutually heterogenic. However, what most cancer cells have in common and what separates them from normal cells is uncontrolled growth, resulting in a high nutritional uptake. An alternative property is the ability to invade normal tissue and metastasize. In this respect, it is not surprising that the potential targets presented in this review support these phenotypic characteristics. The biomarkers reported for CRC can roughly be divided into the following groups:

- Proteins necessary for high cancer cell metabolism and proliferation rate: epidermal growth factor receptor (EGFR), folate receptor-alpha (FR-α), transforming growth factor (TGF), vascular endothelial growth factor (VEGF).
- Proteins with regulatory functions in the extracellular matrix: carbonic anhydrase (CA) IX, collagen, matrix metalloproteinases (MMPs), osteonectin (SPARC).
- Cell adhesion and signaling molecules: cadherin 3, carcinoembryonic antigen (CEA), CD44, CXCR4, epithelial cell adhesion molecule (EpCAM), integrins.
- Cytokines/chemokines and their corresponding receptors, involved in metastasis: CXCR1, CXCR2, CXCR4, CXC chemokine ligands (CXCLs).
- Miscellaneous: cathepsin, inducible nitric oxide synthase (iNOS), mucin 1 (Muc1), neutrophil gelatinase–associated lipocalin (NGAL) also called lipocalin-2 (LCN2), tumor-associated glycoprotein 72 (TAG-72).

These potential targets are summarized in Table 2. As is shown in this table, several potential target candidates can be identified; however, currently, a limited number of matching clinically approved agents are available for application in humans (Table 3).

Some targets have T/N ratio of less than 10. However, it should be noted that some targets internalize the imaging agent more rapidly in tumor cells compared with normal cells [18].

This leads to an accumulation of the conjugated imaging agent, which may compensate the signal for the lower T/N ratio, as with FDG-PET imaging [18,20].

Which Biomarkers Meet the Targeting Criteria?

When applying the proposed TASC score (Table 1) to the biomarkers mentioned in Table 2, not all requirements can be objectified by data from literature. Most often, expression rates and pattern are unknown; therefore, it would be interesting to focus future research on target finding on these aspects. The following six targets have a score greater than 17 points and can therefore be considered most promising in CRC (Table 4): EpCAM (20 points), CXCR4 (20 points), Muc1 (18 points), MMPs (18 points), EGFR (20 points), and CEA (19 points). In this section, we discuss these targets in more detail, including the status of these biomarkers in targeted imaging.

VEGF-A scores 17 points, which implies less potential as a target. However, given the extensive experience in VEGF-A–targeted imaging, this biomarker was, nevertheless, considered to be promising and is therefore given attention in this section.

Epithelial Cell Adhesion Molecule

EpCAM is a cell surface receptor, which is involved in cell adhesion and is expressed on most epithelial cells. EpCAM is upregulated on several epithelial cancers, including CRC. The expression of EpCAM in CRC is more than 80% [21–23]. Paradoxically, a higher expression of EpCAM on tumor cells is associated with increased tumor cell migration [23]. Eder et al. [24] successfully imaged EpCAM-expressing tumors in mice, using an antibody fragment targeting EpCAM conjugated to radionuclide, for PET imaging.

Edrecolomab and catumaxomab are clinically approved antibodies directed at EpCAM (Table 3) and tested for therapeutic use. However, so far, no obvious therapeutic advantage has been reported for these agents [25–28]. To our best knowledge, these antibodies have not yet been used for *in vivo* imaging of EpCAM. When applying TASC to EpCAM; the total score of 20 points comes about as followed: EpCAM is cell membrane bound (5 points), diffusely upregulated (4 points), has a high T/N ratio (3 points), is upregulated in more than 79% of the CRC patients (5 points), has been previously imaged with success *in vivo* (2 points), and is able to internalize a compound (1 point) [21,22,29].

CXC Chemokine Receptor 4

CXCR4 is a cell surface receptor involved in homing of hemopoietic stem cells and lymphocytes to the bone marrow, but it is also

Table 2. Proteins Upregulated in Colorectal Cancer.

TASC Item	I	I	II	III	IV	V	VI	VII	TASC Score	References
	Extracellular: Membrane-bound or Secreted	In Close Proximity of Tumor Cell	Pattern of Up-regulation by Tumor Tissue	T/N Ratio	Percentage Patients with Positive Colorectal Tumors	Previously Imaged	Enzymatic Activity	Target-Mediated Internalization		
CA IX	Membrane-bound	Yes	Focal as well as diffuse	High	~47%	Animal experiment [121,122]	Yes	Yes [123]	11	Niemela et al. [124], Kivela et al. [125], Saarnio et al. [126]
Cadherin 3	Membrane-bound	Yes	Diffuse	High	Probably high; no percentage known	No	Not described	Not described	15	Imai et al. [127]
Cathepsin B, D	Mostly secreted; cathepsin B is partly membrane-bound	Mainly	Diffuse, but most expression at invasion front	High	~60%	Animal experiment [128–130]	Yes	Not described	16	Kaneko et al. [45], Kuester et al. [131], Emmert-Buck et al. [82]
CD44	Membrane-bound	Yes	Diffuse	~1.4	~50%	Animal experiment [132]	Not described	Yes [133]	16	Bendardaf et al. [134], Fernandez et al. [135]
CEA	Partly membrane-bound; partly secreted	Mainly	Diffuse, not homogenous	>60	>90%	In patients [69,136,137]	Not described	Yes [138,139]	19	Li et al. [61], Kim et al. [62], Suwanagool et al. [63], Hamada et al. [64]
COL11A1	Secreted	Yes	Unknown	Unknown	Unknown	No	Not described	Not described	3	Bowen et al. [140]
CXCL 1, 5	Secreted	Unknown	Unknown	High	Unknown	No	Not described	Not described	3	Rubie et al. [179], Wen et al. [141]
CXCR1	Membrane-bound	Yes	Diffuse, mainly in primary tumor	High	~55%	No	Not described	Yes [142,143]	16	Rubie et al. [30]
CXCR2	Membrane-bound	Yes	Diffuse, mainly in primary tumor	High	~60%	No	Not described	Yes [142,143]	16	Rubie et al. [30]
CXCR4	Membrane-bound	Yes	Diffuse, more expression in metastases	High	~70%	Animal experiment [32]	Not described	Yes [144]	20	Rubie et al. [30]
EGFR	Membrane-bound	Yes	Diffuse	Unknown, probably high	~80%	In patients [108] Animal experiment [111,114,145–147]	Not described	Yes [148]	20	Cunningham et al. [101], Goldstein et al. [102]
EpCAM	Membrane-bound	Yes	Diffuse	High (own data)	>79%	Animal experiment [149]	Not described	Yes [29]	20	Paret et al. [21], Xie et al. [22]
Folate receptor-α	Membrane-bound	Yes	Diffuse, little strong expression	High	~40%	In patients [150,151]	Not described	Yes [152]	15	Shia et al. [153]
Galectin 3	Partly membrane-bound, partly secreted	Yes	Diffuse, but not homogenous	High	65%-95%	Animal experiment [154,155]	Not described	Yes [156]	13	Paret et al. [21], Endo et al. [157], Tsuboi et al. [158]
iNOS	Mainly intracellular	Yes	Diffuse	High	~78%	Animal experiment [159]	Yes	Is already intracellular	16	Yu et al. [160], Zafirellis et al. [161]
Integrins	Membrane-bound	Yes	Diffuse	Unknown, but imaging T/N ratio >5 [188]	~60%	In patients [163–164]	Not described	Yes [165,166]	15	Fan et al. [167], Sipos et al. [168]
MMP1, 2, 3, 7, 9	Mainly secreted	Yes	Diffuse	Moderate to high	30%-95%	Animal experiment [88,89,91,169]	Yes	Not described	18	McKerrow et al. [79], Jeffery et al. [80], Madoz-Gurpide et al. [81], Kaneko et al. [45], Emmert-Buck et al. [82]
Muc1	Membrane-bound	Yes	Diffuse, more expression in larger tumors and lymph node metastases	High	~50%	In patients [54–56,60]	Not described	Yes [170]	18	Kaneko et al. [45], Suzuki et al. [46]
NGAL (LCN2)	Secreted	Mainly	Diffuse	High	~75%	*In vitro* [171]	Not described	Not described	15	Conrotto et al. [171], Madoz-Gurpide et al. [81]
Osteonectin (SPARC)	Secreted	Yes	Diffuse	High	High, no percentage known	Animal experiment [172]	Not described	Not described	17	Madoz-Gurpide et al. [81]
TAG-72	Partly membrane-bound; partly secreted	Mainly	Not diffuse	High	46%-98%	In patients [173,174]	Not described	Not described	11	Loy et al. [175], Muraro et al. [176], Molinolo et al. [177]
TGFBI	Secreted	Mainly	Unknown	High	Unknown	No	Not described	Not described	6	Roessler et al. [178]
VEGF-A	Partly membrane-bound, partly secreted	Mainly	Diffuse, more expression in metastases	High	56%-78%	In patients [40,44]	Not described	Not described	17	Cao et al. [38], Abdou et al. [39]

Under "previously imaged" (item V), only the most advanced research is mentioned. For each biomarker, the final TASC score is given, based on the characteristics as explained in Table 1.

Table 3. Clinically Approved Ligands for the Biomarkers Mentioned in Table 2.

Target	Clinically Approved Ligand	In Clinical Trial
CEA	Arcitumomab, Altumomab	
CXCR4	AMD3100	BKT-140, AMD11070, MSX-122
EGFR	Cetuximab, Panitumumab, Nimotuzumab	Necitumumab, Zalutumumab
EpCAM	Edrecolomab, Catumaxomab (anti-EpCAM × anti-CD3)	Adecatumumab, Tucotuzumab
Folate receptor-α	Folate	
Integrin		MoaB PF-04605412 (mAb against $\alpha_5\beta_1$ integrin), Etaracizumab (mAb against $\alpha_v\beta_3$ integrin)
Muc1	Pemtumomab	90Y-hPAM4
TAG-72	Anatumomab mafenatox, Minretumomab	
VEGF	Bevacizumab, Ranibizumab	

associated with metastatic spread in several types of cancer, including CRC. CXCR4 is expressed in approximately 70% of the colorectal tumors [30].

Imaging of CXCR4 has recently attracted attention of many different research groups. Nimmagadda et al. [31,32] reported imaging of CXCR4 in tumor-bearing mice using a radionuclide-labeled anti-CXCR4 monoclonal antibody (mAb) using SPECT/CT scanning. Recently, the same group also succeeded in imaging CXCR4 expressing tumors in mice with the use of AMD3100, a clinically approved molecule that selectively binds to CXCR4 (Table 3), conjugated to a radionuclide [32]. AMD3100 is a clinically approved agent that is most promising in harvesting hemopoietic stem cells from the bone marrow. Alternatively, CXCR4-targeting peptides conjugated to a radionuclide or a fluorophore have been reported [33,34]. Misra et al. [35] labeled stromal-derived factor-1 alpha (SDF-1α), a ligand of CXCR4, to a radionuclide for myocardial infarction imaging purposes.

When applying TASC, CXCR4 is granted 20 points based on its expression in CRC.

Vascular Endothelial Growth Factor-A

VEGF is an epithelial growth factor that is most extensively known for its ability to induce angiogenesis. Angiogenesis in turn is considered one of the primary markers in tumor diagnostics [36]. There are four VEGFs, namely VEGF-A, -B, -C, and -D. VEGF-A is the most important subtype. When tumor cells become hypoxic, VEGF-A expression is upregulated [36]. VEGF-A is partly membrane bound, but it also diffuses through the interstitial cell space. The latter potentially limits broader use as a target. However, the highest VEGF-A concentrations are observed close to the source of expression, inducing the creation of new blood vessels to the hypoxic tumor areas [37].

VEGF-A is upregulated in more than 56% to 78% of all colorectal tumors [38,39].

Multiple groups have successfully imaged VEGF-A expression in tumors induced in animals using a VEGF-A antibody conjugated to an imaging agent. Imaging has most commonly been performed with bevacizumab (Avastin; Roche), a clinically approved therapeutic anti–VEGF-A mAb, which was made suitable for imaging by conjugation to a radionuclide [40–44].

In a clinical imaging study, Scheer et al. [40] did not find a significant correlation between VEGF-A expression and a positive SPECT signal, which may imply that the used tracer was not specific enough. Furthermore, a study in melanoma patients with bevacizumab conjugated to a radionuclide by Nagengast et al. [44] yielded more promising results.

When applying TASC to VEGF-A, a total of 17 points are granted. This low score is mainly caused by the fact that the largest proportion of VEGF-A is not membrane bound and by the expression in a relatively low percentage of patients with CRC. However, because of the recent results in various imaging modalities, as described above, VEGF-A can be considered a potential target for future imaging purposes and is therefore worth to be included in this overview.

Mucin 1

Muc1 is a cell surface receptor that plays a role in protection and lubrication of epithelial surfaces in luminal structures. This receptor is also involved in signal transduction in cell adhesion and antiadhesion mechanisms. Overexpression of Muc1 is often found on malignant cells. In CRC, Muc1 is expressed on approximately 50% of the tumors [45,46].

Different groups have successfully imaged Muc1 in tumor-bearing mice using muc1-targeted monoclonal antibodies or aptamers conjugated to a radiopharmaceutical [47–53]. The use of monoclonal antibodies directed to Muc1 conjugated to a radionuclide has already been described in patients with bladder and pancreatic cancer [54–56]. Medarova et al. [57] described the use of a dual-modality imaging agent by conjugating a Muc1-targeting peptide to fluorophore Cy5.5 for fluorescence imaging and to iron oxide nanoparticles for magnetic resonance (MR) imaging. This probe was tested in mice bearing human pancreatic cancer with good imaging results for both modalities. Muguruma and Ito [58] proved the ability to endoscopically detect tumors by using a fluorescent antibody–based tracer targeting Muc1, in freshly resected specimens of gastric cancer. A different approach for tumor imaging is a two-step pretargeting technique using a bispecific antibody. An antibody directed to both Muc1 and the used radiopharmaceutical is administered on which the radiopharmaceutical is administered subsequently. The radiopharmaceutical binding site of the circulating antibody can be blocked, thus yielding a higher tumor-to-background ratio [59]. Promising results were obtained in breast cancer patients with bispecific antibody-based PET scanning [60].

The total TASC score for Muc1 in CRC is 18 points.

Table 4. The Biomarkers That Score 18 or More Points When Applying TASC.

Biomarker	TASC Score
CXCR4	20
EpCAM	20
EGFR	20
CEA	19
Muc1	18
MMPs	18
VEGF-A	17*

These biomarkers are regarded most promising for tumor-targeted imaging in colorectal cancer.
*VEGF-A scores 17 points but was nonetheless included based on the broad experience with this marker for imaging purposes.

Carcinoembryonic Antigen

CEA is a glycoprotein that plays a role in cell adhesion. In healthy adults, hardly any CEA is found; however, CEA is strongly expressed in CRC (>90%) [61–64] and is one of its best studied tumor markers. CEA is also measurable in blood, but by far, the highest concentration of CEA is found at the tumor site. CEA imaging using a CEA-directed antibody or antibody fragment conjugated to a radionuclide has extensively been described in animal studies and in patients, without showing disadvantages of having simultaneous high serum and tumor CEA levels [65–70]. Yazaki et al. [71] fused CEA-antibody fragments conjugated to a radionuclide to albumin for a more specific tumor uptake. Technetium 99m (^{99m}Tc) arcitumomab is a commercially available antibody fragment directed to CEA conjugated to ^{99m}Tc, which is used in the CEA scan. However, in comparison to FDG-PET, ^{99m}Tc arcitumomab offers little convincing advantages in the detection of CRC [72,73]. The use of CEA-antibody fragment–based radiotracers for guided surgery has also been described [68,70,74].

As well as in Muc1 targeting, the two-step pretargeting system using a bispecific antibody has been described in animal studies and in patients for CEA [75,76].

Few studies are available on fluorescence imaging for targeting of CEA. Fidarova et al. [77] described the use of an anti-CEA mAb conjugated to a fluorophore for the detection of metastatic CRC in mice. Kaushal et al. [78] showed the use of an anti-CEA mAb conjugated to a fluorophore, in intraoperative detection of colorectal tumor deposits, with good *in vivo* results.

When applying TASC to CEA in CRC, the total score is 19 points.

Matrix Metalloproteinases

MMPs are zinc- and calcium-dependent endoproteases that are upregulated in the tumor environment and are capable of degrading proteins in the extracellular matrix. MMPs are upregulated in 30% to 95% of colorectal tumors, depending on the type of MMP (Table 2) [45,79–82].

Several groups have targeted MMPs *in vivo* by using fluorescent or radiolabeled specific MMP-inhibitors [83–86]. One study reports using a radiolabeled mAb for *in vivo* targeting of MMP1, an MMP subtype [87]. Because MMPs have proteolytic activity, this target is ideal for activatable probes. The advantage of activatable probes is that they greatly reduce background signal. Several studies demonstrate the *in vivo* use of proteolytic beacon coupled to a fluorophore, which emits a signal after cleavage by MMP [88,89]. MMPsense is a commercially available MMP-dependent activatable fluorescent probe, successfully tested in *in vivo* models [90]. Veiseh et al. [91] describe the *in vivo* use of chlorotoxin, a small peptide derived from snake venom that interacts with MMP2, conjugated to the fluorophore Cy5.5, for potential intraoperative imaging. Lepage et al. synthesized a contrast agent containing gadolinium chelate, which is cleaved by MMP. On cleavage, this agent is less soluble in water and remains at the tumor site. Good *in vivo* results have been demonstrated for MR imaging using this protease-modulated contrast agent [92–94]. Aguilera et al. [95] developed activatable cell penetrating peptides (ACPPs) that enter the cell after cleavage by MMP. The ACPPs were labeled with Cy5.5 for fluorescence imaging, with gadolinium chelate for MR imaging, or with both for dual imaging [96]. These ACPPs were further improved by conjugation to large molecule dendrimers, which improved tumor uptake and thus the emitted signal [97,98].

MMPs granted an average of 18 points in CRC when applying TASC, depending on the subtype.

Epidermal Growth Factor Receptor

EGFR is a cell surface receptor involved in processes such as cell proliferation, differentiation, adhesion, and migration. EGFR is upregulated in different types of cancer, including skin, breast, ovary, bladder, prostate, kidney, head and neck, and non–small cell lung cancers [99,100]. In colorectal cancer, EGFR is upregulated in approximately 80% of the tumors [101,102].

EGFR has been extensively imaged by radionuclide- or fluorophore-conjugated antibodies. Most often, cetuximab, a clinically approved anti-EGFR antibody, is used [103–107]. In 1994, Dadparvar et al. [108] administered radionuclide-labeled anti-EGFR antibodies to patients with intracranial neoplasms for SPECT scanning. Although promising results were obtained, to our knowledge, no sequel was given to this radiopharmaceutical. Also, a few studies described the use of panitumumab *in vivo*, which is the second clinically approved antibody directed at EGFR [109,110]. Variants using antibody fragments or affibodies have been described in animal studies [111,112].

Alternatively, EGF, the natural ligand of EGFR, is also used *in vivo* as an imaging agent, conjugated to mainly fluorophores or quantum dots [113–115]. Goetz et al. [116] described a fluorescent anti-EGFR antibody capable of imaging human CRC tissue, which is not only successful in *in vivo* imaging results but also potentially useful in endoscopy. Hama et al. [117] described an alternative two-step pretargeting model, using nonfluorescent biotinylated cetuximab as first antibody, followed by a neutravidin-BODIPY-FL fluorescent conjugate. The latter binds to the first antibody by a neutravidin-biotin binding. The concept was tested *in vivo* in a PC model. A 10-fold signal amplification was found, leading to high tumor-to-background ratios and good detection of lesions as small as 0.8 mm.

The TASC score of EGFR in CRC adds up to 20 points.

Discussion

TASC needs to be validated in other cancer types and adjusted where necessary.

It should be pointed out that TASC is designed as a directive which can help gain objectivity and extra insight in target selection. Future validation studies and adjustments, to our opinion, will improve TASC to make it more broadly applicable to various types of cancer. Immunohistochemical analysis of collected tumor specimens is a relatively easy way to determine applicability of a target. In the case of a promising target, further validation is needed by testing a target-directed imaging probe *in vitro*, for proof of concept and specific binding, and, subsequently, in appropriate tumor mouse models *in vivo*.

Expression of a target may depend on tumor stage. For example, CXCR4, EGFR, and VEGF are associated with more advanced tumor stages and metastasis in CRC [118–120]. However, MUC1 is also generally expressed in T1 CRC tumors [46]. Therefore, such a target may also be of value in early CRC detection.

Conclusions

In PC of colorectal origin, tumor-targeted imaging may yield better diagnostic and therapeutic results. A large number of tumor biomarkers are upregulated in CRC. However, there is no objective system for selecting their clinical applicability in targeted imaging applications. In this review, we introduce a novel scoring system for target selection for imaging purposes, the TASC. When applying TASC to

biomarkers for CRC, we found that the most potent targets for imaging are CXCR4, VEGF-A, Muc1, MMPs, EGFR, EpCAM, and CEA based on their scoring. Clearly, the ideal target for imaging purposes does not exist; moreover, by using the TASC system, we propose a novel guideline in tumor targeting for selecting appropriate targets for imaging purposes.

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