Czech Metastatic Colorectal Cancer Patients: their Copy Number Variation and Clinical Response to Chemotherapy and Bevacizumab

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Introduction and aims

Bevacizumab (Avastin®) is a **humanized monoclonal antibody** targeting **vascular endothelial growth factor A** (VEGF-A), inhibiting angiogenesis in **metastatic colorectal cancer** (mCRC) (Fig. 1). It is primarily used in patients with ***RAS* mutations**, where cetuximab or panitumumab are ineffective. Bevacizumab is usually part of the FOLFOX or XELOX combination treatment for CRC in Czech cancer centers. Compared to cetuximab, bevacizumab prolongs overall survival and progression-free survival (PFS) in right-sided *RAS* wildtype and *BRAF* wildtype and *BRAF* mutant tumours [1,2]. Although bevacizumab is established in mCRC treatment, **no definitive DNA biomarker** for its efficacy exists [3]. Somatic mutations in ***NRAS*, *BRAF*,** and/or ***PIK3CA*** have been studied but lack validation as predictive markers [4]. We hypothesize that **somatic copy number variations** (CNVs) might be key biomarkers for bevacizumab efficacy.

Material and methods

Clinical records of 142 patients with mCRC treated at University Hospital Olomouc were used to select **15 + 15 patients** according to their **clinical response** to **bevacizumab treatment** based on **progression-free survival: poor responders** (PFS ≤ 9 months) and **good responders** (PFS ≥ 10 months) (Tab. 1, Tab. 2). **DNA** was isolated from **FFPE samples** using the Cobas DNA Sample Preparation Kit (Roche) and **quantified by qPCR** [5]. **CNV analysis** was performed using the **OncoScan FFPE Assay Kit 1.0** (Thermo Fisher Scientific), which applies molecular inversion probes technology to detect CNVs in FFPE-derived DNA. The data was processed using **OncoScan Console 1.3** (Thermo Fisher Scientific) and **R software** [6] using the **rCGH package** [7] for segmentation and **GISTIC 2** analysis [8]. Functional annotation of genes was done using the **DAVID database version 6.8** [9] and its Functional Annotation Chart tool were used to annotate genes.

Results and discussion

Good responders showed **amplifications** in the **18p11.32** region and **deletions** in chromosomes **1p36.33**, **8p11.22**, **10q11.23**, **14q32.33**, **16p13.3**, and **20p12.1**. **Poor responders** exhibited **amplifications** at **8q24.21**, **14q12**, and **19q13**.2, with **no deletions above** the threshold (Fig. 2).

Functional annotation revealed that **good response** genes were involved in **ATPase activity**, **neuronal signaling**, and **transcription regulation**. **Poor responders** had amplified genes linked to **immune regulation** (*IFNL1*, *IFNL2*, *IFNL3*), ***MAPK* signaling** (*MAP3K10*, *MAP4K1*), and **differentiation** (*EID2*, *SIRT2*). Genes like *AGRN*, *MAPK8*, and *ARHGAP22* from **good responders** have been linked to **angiogenesis** and **treatment resistance**, while *DVL1* and *MYC* in **poor responders** are associated with **tumour proliferation** (Tab. 3).

Conclusion

This study suggests that CNVs detected by OncoScan technology could serve as biomarkers for predicting bevacizumab response in mCRC. Identifying genes linked to angiogenesis, tumourigenesis, and proliferation (e.g., *AGRN*, *MAPK8*, *MYC*, and *DVL1*) requires further validation in larger cohorts. Such findings could lead to personalized therapies, improving mCRC treatment outcomes. Future studies will validate these results using high-throughput methods like PCR to confirm their clinical utility.

Acknowledgement

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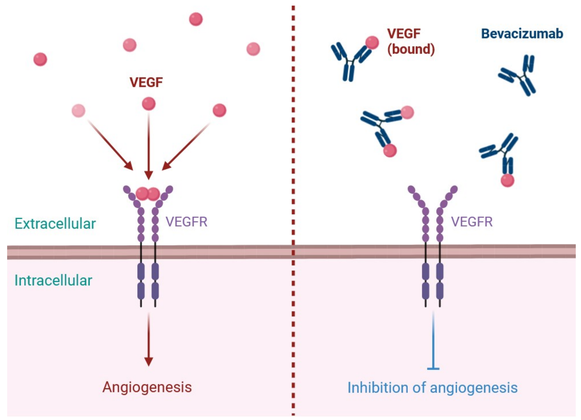

Fig. 1: Bevacizumab inhibits the angiogenic activity of VEGF. Created in https://BioRender.com according to https://www.avastin.com/hcp/mcrc/proposed-moa.html.

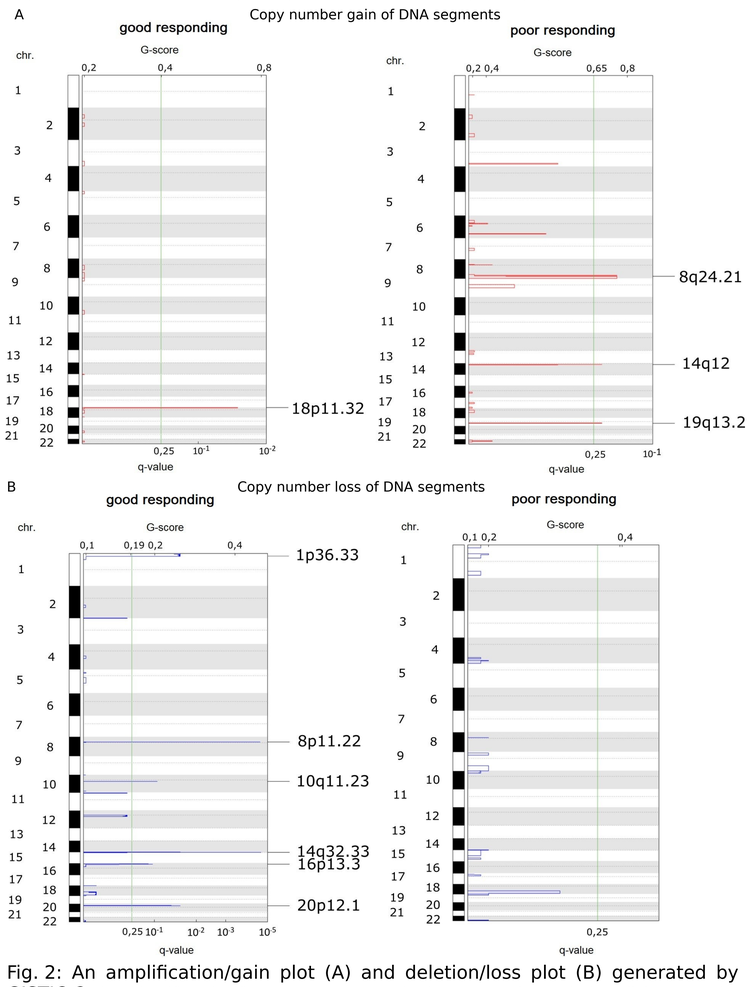

Fig. 2: An amplification/gain plot (A) and deletion/loss plot (B) generated by GISTIC 2 that identifies significant tumour targets in the genome by analysing all features with increased copy numbers of DNA segments within selected regions. The G-score value takes into account the intensity of the aberration as well as the frequency of its occurrence across samples. The q-value = 0.25, illustrated by the green line, represents the significance threshold.

Parameter	Poor Responding Patients	Good Responding Patients
sex	8 F / 7 M	7 F / 8 M
age	42-76 years (median 62 years)	45-70 years (median 62 years)
tumour tissue	10 primary carcinomas / 5 metastasis	7 primary carcinomas / 8 metastasis
colon	3 right / 12 left	3 right / 12 left
therapy length	median 168 days	median 236 days

Tab. 1: Patients cohort parameters.

	Sex	Age at diagnosis	Tumour tissue	Tissue origin	Colon	Therapy length (days)	PFS (months)
Poor Responding Patients							
Patient 1	male	66	meta	rectosigmoid junction	left	98	3
Patient 2	female	64	prim	rectum	left	108	4
Patient 3	male	63	prim	sigmoid colon	left	154	5
Patient 4	female	60	meta	caecum	right	245	8
Patient 5	male	64	prim	ascending colon	right	161	8
Patient 6	male	52	prim	ascending colon	right	111	9
Patient 7	male	61	prim	rectum	left	161	5
Patient 8	female	64	meta	rectum	left	120	5
Patient 9	female	57	prim	rectosigmoid junction	left	168	6
Patient 10	female	56	prim	sigmoid colon	left	181	6
Patient 11	female	62	meta	rectum	left	177	6
Patient 12	female	42	prim	sigmoid colon	left	189	6
Patient 13	male	49	prim	sigmoid colon	left	180	7
Patient 14	male	69	prim	rectum	left	236	7
Patient 15	female	76	meta	splenic flexure	left	184	6
Good Responding Patients							
Patient 16	male	62	prim	rectosigmoid junction	left	301	10
Patient 17	male	65	prim	rectum	left	154	13
Patient 18	female	68	meta	sigmoid colon	left	877	14
Patient 19	male	59	meta	caecum	right	236	14
Patient 20	female	70	meta	caecum	right	739	16
Patient 21	female	65	prim	sigmoid colon	left	113	10
Patient 22	male	68	prim	hepatic flexure	right	245	11
Patient 23	female	49	meta	rectum	left	159	12
Patient 24	male	54	prim	rectum	left	499	12
Patient 25	female	52	prim	rectum	left	351	18
Patient 26	male	45	prim	sigmoid colon	left	238	18
Patient 27	female	65	meta	rectum	left	168	19
Patient 28	male	61	meta	sigmoid colon	left	132	23
Patient 29	male	51	meta	sigmoid colon	left	109	94
Patient 30	female	69	meta	sigmoid colon	left	145	11

Tab. 2: Patient characteristics in both cohorts.

	Altered area	Genes in the area	Frequency of signal
Good responding patients			
ATPases, type AAA	1p36.33	ATAD3A, ATAD3B, and ATAD3C	7/15
Neuronal signal transmission	1p36.33 10q11.23	AGRN and DVL1 MAPK8, CHAT, and SLC18A3	7/15 2/15
Regulation of transcription	10q11.23 18p11.32	ERCC6 THOC1	2/15 3/15
Superior domain PH type	1p36.33 10q11.23	ACAP3 and PLEKHN1 AGAP4, ARHGAP22, and WDFY4	7/15 2/15
Poor responding patients			
Galectines	19q13.2	CLC, LGALS13, LGALS17A, LGALS14, LGALS16, LGALS4, LGALS7, and LGALS7B	8/15
Jak-STAT signalling pathway	19q13.2	IFNL1, IFNL2, and IFNL3	8/15
MAPK cascade	19q13.2 8q24.21	MAP3K10, MAP4K1, ZFP36, PSMC4, PSMD8, and RASGRP4 MYC	8/15 12/15
Differentiation	19q13.2	EID2, EID2B, SIRT2, CATSPERG, DLL3, and GGN	8/15
F-box associated domain	19q13.2	FBXO17, FBXO27, and NCCRP1	8/15

Tab. 3: Significant functional groups of genes overview.

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