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Original Research

AXL is a predictor of poor survival and of resistance to anti-EGFR therapy in RAS wild-type metastatic colorectal cancer

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Abbreviations: CMS, Colon molecular subtype; EGFR, Epidermal growth factor receptor; FFPE, Formalin-fixed paraffin-embedded; GEO, Gene expression omnibus; HNSCC, Head and neck squamous-cell carcinoma; mCRC, Metastatic colorectal cancer; MSS, Microsatellite stable; moAb, Monoclonal antibodies; NSCLC, Non–small-cell lung cancer; OS, Overall survival; PFS, Progression-free survivall; PD, Progressive disease; 3-D, Three-dimensional; WT, Wild type.

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Abstract ***Background:*** RAS mutations are the only validated biomarkers in metastatic colorectal cancer (mCRC) for anti-epidermal growth factor receptor (EGFR) therapy. Limited clinical information is available on AXL expression, marker of epithelial to mesenchymal transition, in mCRC.

Methods: AXL was retrospectively assessed by immunohistochemistry in 307 patients. RAS wild-type (WT) patients ($N = 136$) received first-line anti-EGFR–based therapy; RAS mutant patients ($N = 171$) received anti-angiogenic–based regimens.

Preclinical experiments were performed using human RAS WT CRC cell lines and xenograft models. AXL RNA levels were assessed in a cohort of patients with available samples at baseline and at progression to anti-EGFR treatment and in the GSE5851 dataset.

Results: AXL was expressed in 55/307 tumour tissues, correlating with worse survival in the overall population (AXL-positive, 23.7 months; AXL-negative, 30.8 months; HR, 1.455, $P = 0.032$) and in RAS WT patients (AXL-positive, 23.0 months; AXL-negative, 35.8 months; HR,1.780, $P = 0.032$). Progression-free survival (PFS) in the RAS WT cohort was shorter in the AXL-positive cohort (6.2 months versus 12.1 months; HR, 1.796, $P = 0.013$). Three-dimensional cultures obtained from a patient following anti-EGFR therapy resulted AXL-positive, showing resistance to anti-EGFR drugs and sensitivity to AXL inhibition. AXL transfection in CRC cell lines induced AXL overexpression and resistance to the EGFR blockade. At progression to cetuximab, 2/10 SW48-tumour xenograft mice showed AXL expression. Consistently, AXL RNA levels increased in 5/7 patients following anti-EGFR therapy. Moreover, in the GSE5851 dataset higher AXL RNA levels correlated with worse PFS with cetuximab in KRAS-exon2 WT chemorefractory patients.

Conclusions: AXL is a marker of poor prognosis in mCRC with consistent clinical and preclinical evidences of involvement in primary and acquired resistance to anti-EGFR drugs in RAS WT patients.

1. Introduction

Multimodal treatment strategies have significantly improved the prognosis of metastatic metastatic colorectal cancer (mCRC) patients [1]. However, the identification of predictive biomarkers of response remains a major clinical challenge. Currently, the only validated biomarker for anti-epidermal growth factor receptor (EGFR) therapy is represented by the absence of somatic RAS mutations [1]. Although several additional molecular alterations have been associated with resistance or with sensitivity to anti-EGFR monoclonal antibodies (moAb) treatment, their use is not yet incorporated into clinical practice [2].

In the last decade, AXL signalling has been associated with tumour progression, immune suppression, angiogenesis, and epithelial to mesenchymal transition (EMT) in solid tumours [3]. AXL is member of the TAM (TYRO3, AXL, MER) receptor tyrosine kinase family. As per the molecular subtyping consensus classification, AXL signalling is activated in colon molecular subtype 4 tumours, characterised by mesenchymal features and poor prognosis [4]. Limited clinical information is available on AXL expression in CRC [5,6].

The aim of this study was to investigate the role of AXL in mCRC, particularly as a potential mechanism of resistance to anti-EGFR treatment.

2. Methods

2.1. Patients population

AXL expression was retrospectively assessed in formalin-fixed, paraffin-embedded (FFPE) tissues from 307 pretreatment specimens (298 primary sites; 9 metastases), from three patients series (overall population $N = 307$), within clinical trials in different institutions [7–10]. (Patient population is detailed in Supplementary Figure A1 and Table A1). All patients, included in the analysis upon informed consent for translational research, received first-line treatment per individual

study protocol: RAS wild-type (WT) patients ($N = 136$) received chemotherapy plus anti-EGFR therapy (cetuximab or panitumumab), whereas RAS mutant patients ($N = 171$) received chemotherapy plus anti-angiogenic therapy (bevacizumab or aflibercept). Progression-free survival (PFS) was defined as the time between treatment initiation and progressive disease (PD) or death from any cause; overall survival (OS) was defined as the time between treatment initiation and death from any cause.

2.2. Immunohistochemistry

AXL expression was assessed by immunohistochemistry in FFPE blocks by two independent investigators (Supplementary Methods). Tumour samples were evaluated in tumour cells and stroma, scored as negative (<1% stained cells) or positive (≥1% cells). Positive cases were graded per staining intensity as weak, moderate or intense.

2.3. Cell cultures, transfection and tumour-xenografted mice

AXL gene transfection was performed in human CaCo-2, LIM1215 and SW48 CRC cell lines and, after assessment of protein expression, treated with cetuximab (Supplementary Methods).

Ten immunodeficient mice bearing human SW48 CRC tumour xenografts were treated with cetuximab until progression (Supplementary Methods).

2.4. RNA analysis from a cohort of patients treated with anti-EGFR therapy

AXL expression was assessed in RNA from paired baseline and post-progression samples from seven patients with RAS WT mCRC treated with anti-EGFR moAbs (cetuximab or panitumumab), as previously described (Supplementary Methods) [11].

2.5. In silico dataset

The gene expression profile data GSE5851 were retrieved from Gene Expression Omnibus (GEO) database performed with the GPL571 platform, using two AXL probes (n.1 = 202,685_s_at and n.2 = 202,686_s_at]. This cohort included 80 chemorefractory mCRC patients enrolled in a phase II clinical trial in which transcriptional profiling was conducted on metastatic biopsies before cetuximab exposure [12]. All patients had annotated information about PFS, defined as the time from study enrolment to PD or death. Of note, information about KRAS exon2 status was available for 70 patients [12].

2.6. Statistical analysis

Differences between categorical data were measured by the χ-square and Fisher exact test, when appropriate. Survival curves were plotted using the Kaplan–Meier method and compared using the log-rank test. The Cox proportional hazards model was used for univariate and multivariate analyses to assess the independence of prognostic factors. Standard error, for both univariate and multivariate model, was calculated using as variance estimators, the bootstrap method. Package survminer (R software) was used to select the best cut-off of AXL levels in the GSE5851 data set; moreover, median value of expression of each probe was used. Quantitative data were reported as mean ± standard deviation and analysed with one-way analysis of variance (ANOVA). Repeated measurements were analysed with Wilcoxon signed-rank test. All analyses were two-sided, with P value < 0.05 indicating statistical significance. Analyses were performed using the SPSS package (v.24), STATA 14 and R (3.6.0).

3. Results

3.1. AXL expression in patients with mCRC and clinical outcome

AXL expression was assessed by immunohistochemistry in the pretreatment tumour samples from 307 patients, 136 with RAS WT tumours and 171 RAS mutant (patient population detailed in Supplementary Figure A1 and Table A1). AXL was expressed in ≥1% of cancer cells in 55 of 307 samples (18%) with different intensity: 40 weak, 8 moderate and 7 intense staining, respectively (Fig. 1A and B). No difference was observed among RAS subgroups. In particular, in the RAS WT cohort, 22 of 136 cases (16%) were positive, whereas in the RAS mutant cohort 33 of 171 were positive (19%) (P = 0.48), with no difference in intensity grade (Fig. 1B). Tumour-associated stroma was assessable in 296 samples. AXL was expressed in 245 of 296 cases (83%). No differences were observed between the RAS cohorts: 111 of 129 (86%) in RAS WT and 134 of 167 (80%) in RAS mutant subgroups were positive for AXL expression, respectively ($P = 0.19$) (Fig. 1E–D). AXL staining mostly displayed membrane positivity. No correlation was found between patients clinico-pathologic features and AXL expression (Supplementary Table A2 and Table A3). Moreover, no significant correlation was found with p53 mutational status (Supplementary Contents).

AXL expression was correlated with patient clinical outcome. In the overall population, AXL expression in tumour cells was associated with a significantly worse median OS, 23.7 months (95%CI, 18.6–28.9) in AXL-positive versus 30.8 months (95%CI, 27.2–34.3) in

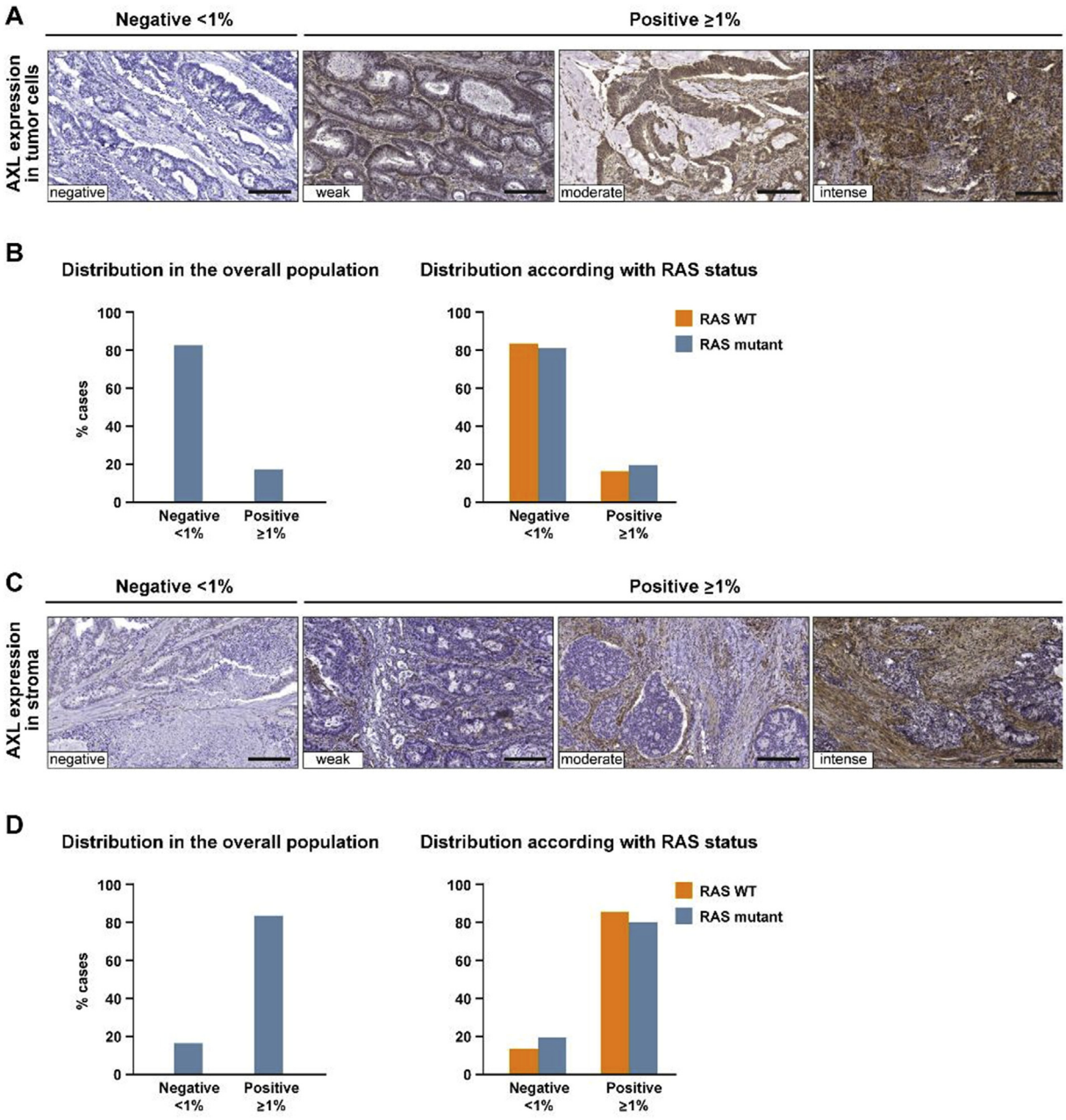

Fig. 1. **AXL expression in tumour cells and tumour-associated stroma. (A)** AXL expression in tumour cells by immunohistochemistry scored as negative (<1% stained cells) and positive (≥1% stained cells), graded per intensity as weak, moderate and intense. Magnification 10×, 200 μm scale. **(B)** Distribution of AXL expression in the overall population and between RAS subgroups. **(C)** AXL expression in tumour-associated stroma by immunohistochemistry scored as negative (<1% stained cells) and positive (≥1% stained cells), graded per intensity as weak, moderate and intense. Magnification 10×, 200 μm scale. **(D)** Distribution of AXL expression in tumour-associated stroma in the overall population and between RAS subgroups.

AXL-negative patients, Hazard Ratio (HR) = 1.455 (95%CI, 1.032−2.050), $P = 0.032$ (Fig. 2A). Stratifying the population based on the RAS status, in the RAS WT cohort a negative prognostic effect was observed with a median OS of 23.0 (95%CI, 13.7−32.3) versus 35.8 months (95%CI, 29.2−42.2), HR = 1.780 (95%CI, 1.050−3.015), $P = 0.032$. This difference did not reach statistical significance in the RAS mutant (Fig. 2B and C).

We next investigated the association between AXL expression and first-line treatment activity. In RAS WT patients treated in first-line with anti-EGFR drugs with chemotherapy, antitumour activity was significantly lower in patients with AXL-positive tumours, with a median PFS of 6.2 months (95%CI, 4.2−8.2) versus 12.1 months (95%CI, 10.6−13.6), HR = 1.796 (95%CI, 1.131−2.851), $P = 0.013$ (Fig. 2D). In the RAS mutant group, treated with anti-angiogenic agents in

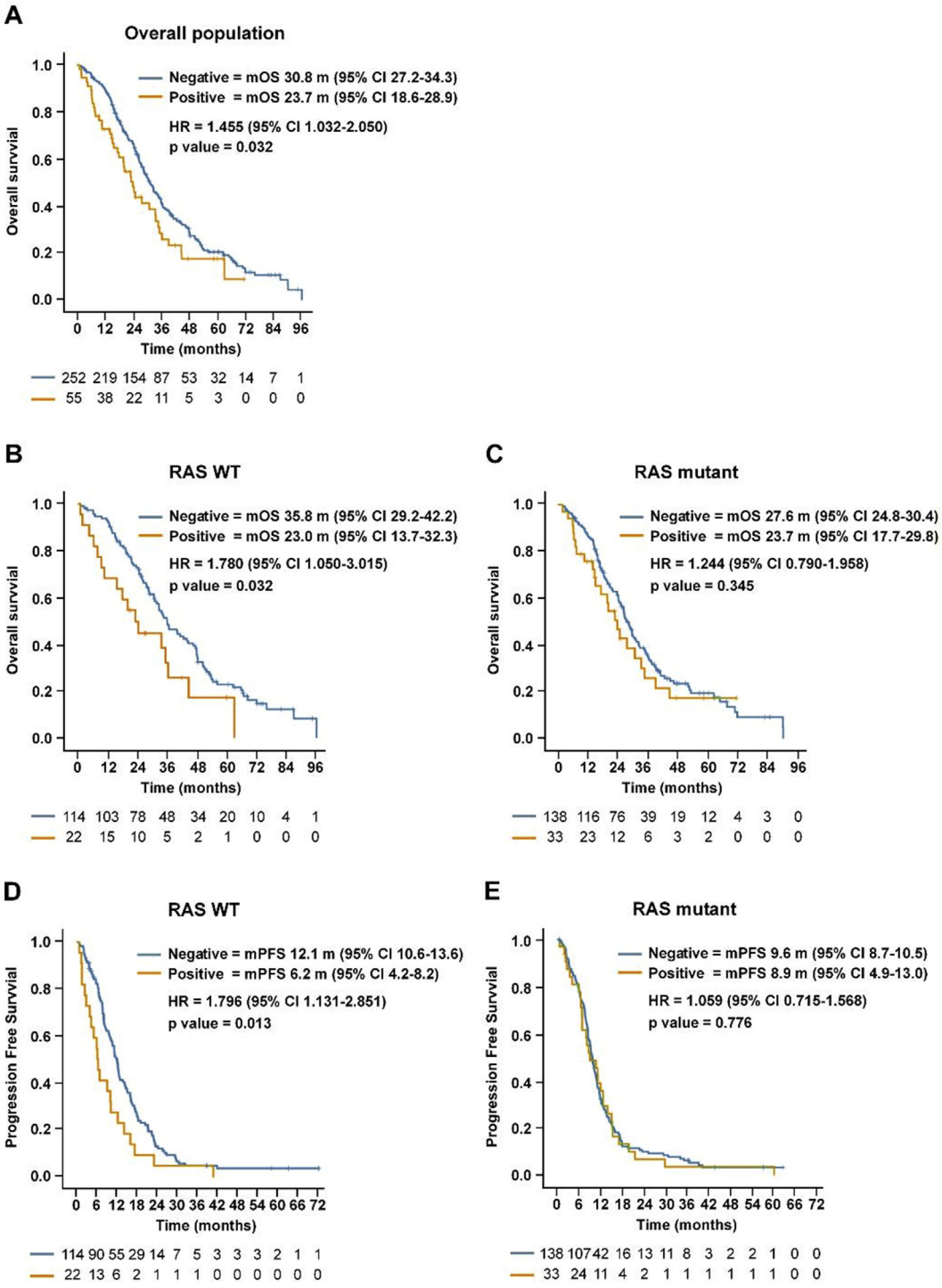

Fig. 2. **AXL expression in tumour cells and clinical outcome. (A)** Kaplan–Meier of overall survival (OS) in accordance with AXL expression in the overall population. **(B)** Kaplan–Meier of OS in accordance with AXL expression in RAS WT and RAS-mutant cohorts. **(C)** Kaplan–Meier of progression-free survival (PFS) in accordance with AXL expression in RAS WT and RAS-mutant cohorts.

combination with chemotherapy, no impact of AXL expression was observed (Fig. 2E).

The potential prognostic effect of AXL expression in tumour stroma was also investigated. In the overall population, patients with AXL-positive tumour stroma had a worse survival with 28.5 (95%CI, 25.0–31.9) versus 37.7 months (95%CI, 25.6–49.8) in negative cases, HR = 1.533 (95%CI, 1.067–2.202), $P = 0.021$ (Supplementary Figure A2A). No significance was observed after stratification based on RAS status, although in both RAS cohorts a trend in worse outcome was registered (Supplementary Figure A2B-C).

Similarly, no difference in PFS was observed (Supplementary Figure A2D-E). All the statistical analyses were replicated and validated based on the bootstrap method (Supplementary Table A4).

Furthermore, we assessed AXL expression in a patient with RAS and BRAF WT mCRC, with prolonged clinical benefit after treatment with chemotherapy plus cetuximab. At baseline, the tumour sample was negative for AXL expression in tumour cells and positive in stroma, whereas, at progression, the biopsy of a liver metastasis, tumour cells and stroma displayed AXL positivity. The 3-D cultures obtained from the post-progression biopsy following cetuximab were resistant to anti-EGFR treatments and were growth-inhibited by the selective AXL inhibitors TP-0903 and R-428 (BGB324, bemcentinib) (Supplementary Figure A3, details in Supplementary Contents).

3.2. AXL transfection in CRC cell lines

To define the role of AXL overexpression in the acquisition of resistance to anti-EGFR drugs, three human RAS WT CRC cell lines, sensitive to EGFR blockade (Caco-2, LIM1215 and SW48) were transfected with an AXL expression vector. Transfection resulted in the generation of cells overexpressing AXL protein, as shown in Fig. 3A and B. Treatment with cetuximab for 72 h determined little or no growth inhibition in Caco-2-AXL, LIM1215-AXL and SW48-AXL (Fig. 3C).

3.3. AXL expression in SW48 tumour–xenografted mice following anti-EGFR therapy

We next injected human SW48 CRC cells subcutaneously in the dorsal flank of a group of 10 immunodeficient mice. Mice were continuously treated with cetuximab. Following tumour growth inhibition, tumour progression was observed in all cetuximab-treated mice within 8–10 weeks of therapy. At progression, SW48 tumours were collected. AXL protein expression, determined by western blot, was detected in 2 of 10 cases (Fig. 3D). AXL expression (mRNA) levels showed mild variations between the samples, with the exception of sample 7 and 9, whose mRNA levels were significantly increased (200-fold for #7 and 60-fold for #9) (Supplementary Figure A4). Moreover, the Next Generation Sequencing (NGS) analysis performed detected KRAS mutations in 3 of 10 SW48 tumours, not overlapping with AXL-positive cases (Supplementary Materials and Table A5).

3.4. AXL expression in paired biopsies of mCRC patients following anti-EGFR treatment

To further explore the role of AXL in the acquisition of cancer cell resistance to anti-EGFR therapies, AXL RNA expression was evaluated in paired biopsies (baseline and PD) in seven RAS WT mCRC patients, treated with anti-EGFR moAbs (cetuximab or panitumumab) as first-line (M5-M6-M7 cases), as second-

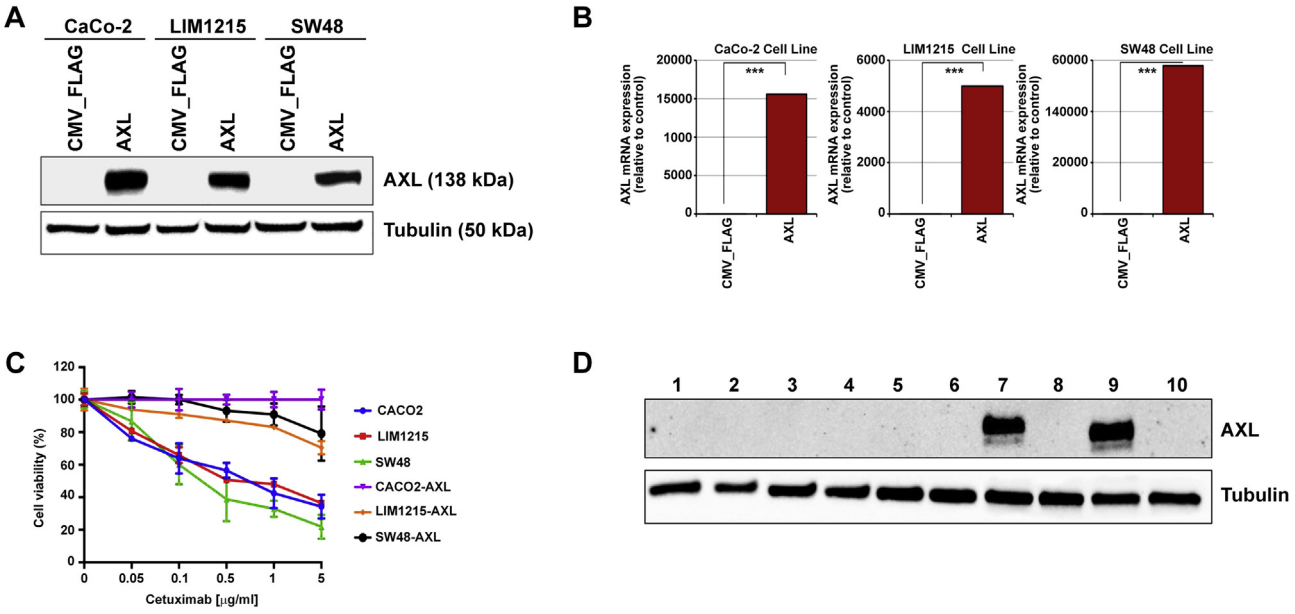

Fig. 3. **AXL as a marker of resistance to anti-EGFR treatment. (A–B)** AXL protein (4B) and AXL mRNA expression (4C) in CRC cell lines (Caco-2, LIM1215 and SW48) transfected with CMV-flag (control plasmid) and with the AXL vector. **(C)** Cell proliferation assay for parental and AXL-transfected CRC cell lines. Parental cell lines exhibited a strong sensitivity to cetuximab at low concentrations (IC50 < 1 ug/ml), whereas AXL-transfected cell lines were resistant to cetuximab. **(D)** Western blot analysis from 10 SW48 tumours xenografted mice. Upon onset of resistance to cetuximab treatment, 2 out of 10 cases exhibited AXL protein expression. Tubulin was used as an internal control. *: $p < 0.05$; ***$p < 0.001$; ****: $p < 0.0001$. CRC, colorectal cancer.

line (M2-M9 cases), or as third-line (M3-M8 cases) therapies. In five of seven cases, AXL RNA expression was increased in post-treatment samples, as compared to baseline values. This difference between pre-treatment and post-treatment AXL RNA levels, expressed as fold-change, appears to be significant (discrepancy value, 1.366 [95%CI, 0.122–5.451], P = 0.0039) (Fig. 4A and B).

3.5. AXL expression from an in silico data set

AXL RNA levels were assessed in a cohort of 80 patients, available in a public microarray tumour data set, retrieved from the GEO database (GSE5851). Chemorefractory mCRC patients were treated with cetuximab monotherapy within a phase II clinical trial and mRNA tumour expression data were obtained by pretreatment biopsies of a metastatic site. Data from two AXL probes were available [12]. Two cut-off values were explored. In the KRASexon2 WT group (N = 70), a difference in cetuximab activity was observed between patients with AXL high or low expression, although this difference did not reach significance, probably due to the small cohort-size. In particular, by using data derived from probe n.1 with a best cut-off approach, in patients with AXL high, PFS was 1.9 months (95%CI, 1.8–2.0) versus 4.1 months (95%CI, 3.9–4.3) in patients with AXL low, HR = 1.548 (95%CI, 0.825–2.906), $P = 0.163$ (Table 1 and Supplementary Figure A5).

4. Discussion

AXL expression has been associated with EMT, tumour growth, invasiveness and drug resistance in solid tumours. However, its relevance in CRC is largely unknown. In the present study, AXL expression was retrospectively assessed by immunohistochemistry in archival pretreatment samples of 307 patients with mCRC. AXL expression in tumour cells was found in 18% of cases. Moreover, 83% of cases presented AXL expression in tumour-associated stroma, a critical component of the tumour-microenvironment, involved in tumour progression through genetic and epigenetic modifications. We also assessed the potential role of AXL on clinical outcome. Previous findings have suggested a role for AXL expression as the predictor of poor survival in early-stage CRC [6]. The results of the present study demonstrate that AXL expression in tumour cells is associated with a worse survival in a large series of mCRC patients, treated in first-line with standard chemotherapy in combination with targeted agents. Moreover, we provide evidence that AXL expression in tumour cells is a biomarker of poor survival in RAS WT mCRC patients. Interestingly, AXL expression in tumour cells is also a marker of lack of efficacy for anti-EGFR therapies in patients with RAS WT mCRC. Of note, no impact on clinical activity in accordance with AXL tumour expression was observed in RAS-mutant mCRC patients treated in first-line with chemotherapy plus anti-angiogenic drugs. In line with these findings, AXL expression has been demonstrated

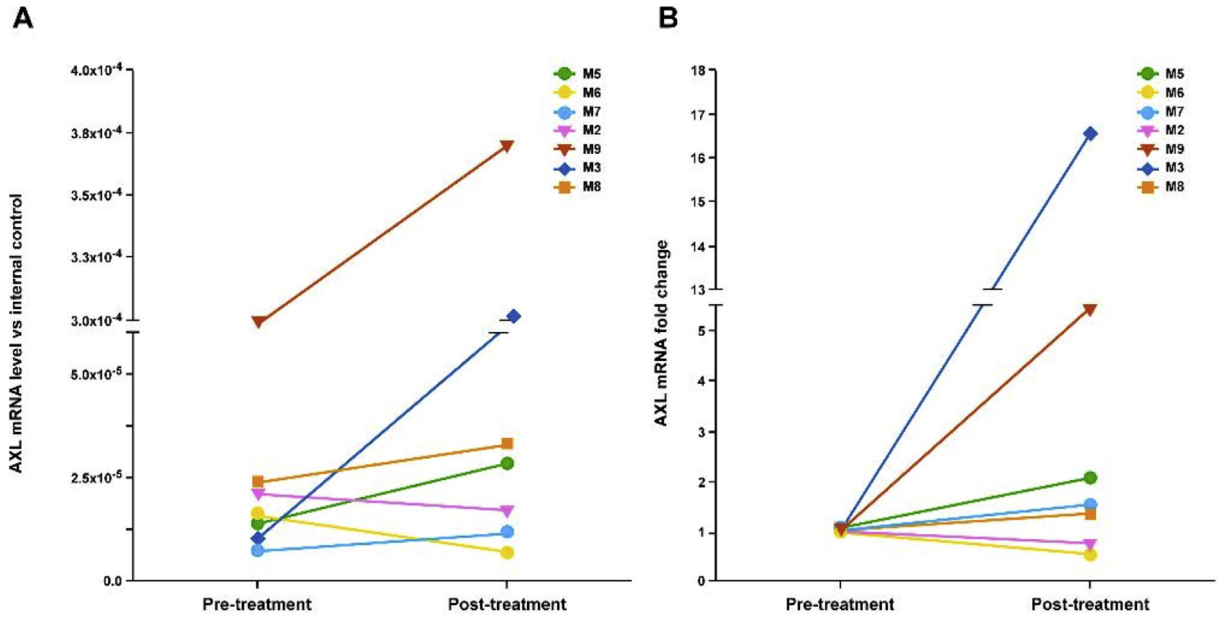

Fig. 4. **AXL expression in two additional series of patients treated with anti-EGFR in different lines of treatment. (A–B)** AXL expression in paired biopsies taken at baseline and after progression to anti-EGFR treatment in different lines. In 5 out of 7 cases, AXL expression levels were increased after treatment. AXL expression was presented as mRNA levels compared to the internal control (18S) (4A) and as mRNA fold-change in post-treatment samples (4B).

Table 1
Progression-free survival (PFS) in accordance with AXL pre-treatment expression in the GSE5851 *in silico* data set of mCRC patients treated with anti-EGFR monotherapy in late-line. Two probes were available; the median value and best cut-off were used as threshold.

AXL RNA expression	KRAS ex2 WT			KRAS ex2 mutant			KRAS ex2 NA		
	Low	High	P value	Low	High	P value	Low	High	P value
Probe n.1 cut off 95.7 months 95% CI	2.2 (0.0–5.5)	1.9 (1.7–2.1)	0.848	2.0 (1.7–2.2)	2.0 (1.9–2.0)	0.182	1.7 (0.3–3.2)	1.9 (0.0–4.0)	0.275
Probe n.1 cut off 58.5 months 95% CI	4.1 (3.9–4.3)	1.9 (1.8–2.0)	0.163	2.1 (1.9–2.3)	2.0 (1.9–2.0)	0.115	2.1 (0.0–2.4)	2.1 (0.0–4.4)	0.125
Probe n.2 cut off 1465.7 months 95% CI	3.8 (0.7–7.0)	1.9 (1.8–2.0)	0.586	2.0 (1.7–2.2)	2.0 (1.9–2.0)	0.665	1.9 (1.6–2.3)	1.9 (1.6–2.3)	0.721
Probe n.2 cut off 1767.8 months 95% CI	3.8 (1.4–6.3)	1.9 (1.8–2.0)	0.474	2.0 (1.7–2.2)	1.9 (1.9–2.0)	0.084	1.9 (1.6–2.3)	1.9 (1.6–2.3)	0.721

CI, confidence interval; ex, exon; WT, wild type; NA, not available

in patient-derived xenografts from surgically resected head and neck squamous-cell carcinoma (HNSCC) with intrinsic resistance to anti-EGFR agents (cetuximab), and AXL expression has been correlated with no response and early relapse to anti-EGFR inhibitor treatment in non–small-cell lung cancer (NSCLC) [13–15]. Moreover, AXL expression has also been associated with acquired resistance in both HNSCC and NSCLC and its inhibition restored sensitivity to anti-EGFR treatment [13,16]. In this respect, we provide experimental evidence to further support these findings. In fact, human RAS WT CRC cell lines, sensitive to anti-EGFR growth inhibition (Caco-2, LIM1215 and SW48), transfected with an AXL expression vector resulted resistant to cetuximab-induced growth inhibition. Furthermore, the clinical case of a patient with RAS and BRAF WT mCRC, with prolonged clinical benefit after treatment with chemotherapy plus cetuximab, supports the evidence of a role for AXL expression as a determinant of acquired resistance to anti-EGFR therapies. In fact, at baseline the tumour sample was negative for AXL expression in tumour cells, whereas in a post-progression biopsy of a liver metastasis, tumour cells displayed AXL positivity. Of interest, the 3-D cultures obtained from the post-progression biopsy following cetuximab were resistant to anti-EGFR treatments and were growth-inhibited by the selective AXL inhibitors TP-0903 and R-428 (BGB324, bemcentinib) [17,18]. Moreover, a role for AXL as a determinant for acquired resistance to anti-EGFR drugs was also indicated by the emergence of AXL protein expression in 2 of 10 SW48 tumour xenografts in immunodeficient mice at progression during treatment with cetuximab.

Finally, to further validate the role of AXL as a marker of lack of efficacy to anti-EGFR therapies, we assessed its expression in two additional series of patients treated with cetuximab or panitumumab in different lines. First, AXL RNA expression was assessed in paired baseline samples and post-progression biopsies of a small cohort of RAS WT mCRC patients, who received anti-EGFR therapies. A difference between paired baseline and post-progression AXL expression was observed. In particular, in five of seven cases, AXL levels significantly increased, suggesting that AXL might be associated with the occurrence of acquired resistance. Second, the data retrieved from the GSE5851 data set [12] suggest that high AXL levels, assessed in biopsies of metastases obtained before cetuximab treatment, are associated with a worse PFS. Within the limitation of the *in silico* approach, of the small sample size and of the molecular assessment limited to KRAS exon2, these findings further support a role for AXL expression in primary resistance to anti-EGFR drugs.

5. Conclusions

This study suggests that AXL is a relevant marker of poor prognosis in mCRC and is associated with shorter PFS in patients with RAS WT mCRC. In this respect, we provide experimental and clinical evidence of AXL as a biomarker of both primary and acquired resistance to anti-EGFR therapies in RAS WT mCRC.

The main limitations to acknowledge for this study are the retrospective nature of the analyses and the small size of the clinical cohort supporting the exploratory translational findings. Thus, our exploratory investigations need a validation into prospective clinical trials. Nevertheless, AXL is a promising target for the development of novel molecular therapeutic strategies in RAS WT mCRC.

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Conflict of interest statement

TT reports advisory board for Amgen, Bayer, Merck, Novartis, Roche and Sanofi.

FM reports advisory board for Lilly and MSD.

ChCr reports personal fees from Roche, Amgen, Bayer and Servier, research funding from Merck Serono and consulting or advisory role for Roche, Bayer and Amgen.

FP reports honoraria/speaker bureau from Amgen, Roche, Merck Serono, Lilly, Sanofi, Bayer and Servier and research grants from BMS.

GWP reports personal fees from advisory boards and/or speaker's honorarium: Amgen, Bayer, BMS, Boston Biomedical, Celgene, Halozyme, Lilly, Merck Serono, Roche, Sanofi, Servier, Shire, MSD, Taiho and CECOG.

NN reports personal financial interests as speaker's fee and/or advisory boards from MSD, Qiagen, Bayer, Biocartis, Incyte, Roche, BMS, Merck, Thermo Fisher, Boehringer Ingelheim, AstraZeneca, Sanofi and Eli Lilly; institutional financial interests (financial support to research projets) from Merck, Sysmex, Thermo Fisher, Qiagen, Roche, AstraZeneca and Biocartis; non-financial interests: President, International Quality Network for Pathology (IQN Path), President Elect, Italian Cancer Society (SIC).

EvMa reports personal financial interests honoraria for advisory role for advisory board from: Astra Zeneca, Sanofi Genzyme, Servier, Celgene, Merck, Lilly and Roche; research grants from MSD and BSD; travel grants from Merck and Roche; institutional financial interests as financial support for clinical trials from Roche, Sanofi Genzyme, Teva and Merck.

GA reports personal financial interests from Hoffman-La Roche, Bristol Myers Squibb, Bayer, Servier, Amgen, Merck Serono and Menarini; institutional financial interests from Bayer, Servier, Novartis, Boehringer Ingelheim, Boston Pharmaceuticals, Hoffman-La Roche and Genentech.

EE reports personal financial interests, honoraria for advisory role, travel grants, research grants from Hoffman-La Roche, Sanofi Aventis, Amgen, Merck Serono and Servier, MSD; institutional financial interests from Hoffman-LaRoche, Sanofi Aventis, Amgen, Merck Serono, MSD, Boehringer Ingelheim, AbbVie, Array Pharmaceuticals, Pierre Fabre and Novartis.

AF reports personal financial interests (advisory role/honoraria): Bayer, Bristol, Lilly, Merck, Pierre Fabre, Roche, Servier; institutional financial interests as financial support for clinical trials from AstraZeneca, Bayer, Bristol, Lilly, Merck, MSD, Novartis, Roche, Sanofi and Servier.

JT reports scientific consultancy roles for Bayer, Boehringer Ingelheim, Chugai, Genentech, Inc., Ipsen, Lilly, MSD, Merck Serono, Merrimack, Merus, Novartis, Peptomyc, Pfizer, Rafael Pharmaceuticals, F. Hoffmann-La Roche Ltd, Sanofi, Seattle Genetics, Symphogen and Taiho.

FC reports being in the advisory boards for Merck, Roche, Amgen, Bayer, Servier, Symphogen and Pfizer and research funding from Roche, Merck, Amgen, Bayer and Ipsen.

ErMa reports being in the advisory boards for Amgen, Bayer, Merck, Roche, Sanofi and Servier and giving expert opinion for ESMO (European Society of Medical Oncology).

ClCa, BB, VMV, GM, VS, PPV, DC, VB, NM, FZ, MD, AN, AB, CA, GS, RF and MS report no competing conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2020.07.010.

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