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Growth differentiation factor 15 (GDF15) predicts relapse free and overall survival in unresected locally advanced non-small cell lung cancer treated with chemoradiotherapy

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Abstract

Introduction Growth differentiation factor 15 (GDF15) is a cytokine of the TGFβ family. Here, we analyzed GDF15 levels in patients with locally advanced non-small cell lung cancer (LA-NSCLC) who participated in OCOG-ALMERA (NCT02115464), a phase II randomized clinical trial, that investigated metformin in combination with standard of care concurrent chemoradiotherapy (cCRT). OCOG-ALMERA was not able to demonstrate benefit in the metformin arm. Therefore, biomarker studies are needed to better define stratification parameters for future trials.

Methods Patients were randomized to treatment with platinum-based chemotherapy and concurrent chest radiotherapy (60–66 Gy), with or without metformin (2000 mg/d). The trial collected tumor volume parameters, survival outcomes, and patient blood plasma at baseline, during (weeks 1 and 6) and 6 months after cCRT. Plasma GDF15 levels were assayed with the ELISA method. Statistical analyses explored associations between GDF15, survival outcomes, and radiotherapy tumor volumes.

Results Baseline plasma levels of GDF15 were elevated in study patients, they increased during cCRT ($p < 0.001$), and the addition of metformin was associated with a further increase (week 6, $p = 0.033$). Baseline GDF15 levels correlated with the radiotherapy gross target volume (GTV, $p < 0.01$), while week 1 of radiotherapy levels correlated with radiotherapy planned target volume (PTV, $p < 0.006$). In multivariate analysis, baseline plasma GDF15 was prognostic for poor relapse-free (RFS) and overall survival (OS) ($p = 0.005$ and $p = 0.002$, respectively).

Conclusions GDF15 is a plasma marker that responds to the treatment of unresected LA-NSCLC with cCRT and metformin. GDF15 levels correspond with tumor volume and increased GDF15 levels predict for poor RFS and OS. These results require validation in larger clinical trial datasets.

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Highlights

- Analysis of plasma specimens of OCOG-ALMERA, a phase II clinical trial that investigated metformin in combination with standard of care concurrent chemoradiotherapy, suggests that the cytokine growth differentiation factor 15 (GDF15) may be a promising biomarker in unresected locally advanced non-small cell lung cancer (LA-NSCLC).
- GDF15 levels increased during chemoradiotherapy, did more so in metformin-treated patients and corresponded with the radiotherapy clinical target volumes.
- Baseline plasma levels of GDF15 predicted overall and relapse-free survival. Validation of these results within larger clinical trials could lead to the establishment of an easily assessable plasma biomarker that could guide LA-NSCLC treatment in the future.

Keywords Lung cancer biomarker, GDF15, Concurrent chemoradiotherapy, Metformin

Introduction

Non-small cell lung cancer (NSCLC) represents 85% of all lung cancer cases [1, 2] and is frequently diagnosed at a locally advanced (LA) stage that is inoperable and has a poor prognosis after standard of care treatment with concurrent chemoradiotherapy (cCRT) [3–5]. In recent years, consolidation immune checkpoint inhibitor therapy has been added to cCRT, but this provided only moderate improvements in survival [6, 7].

Earlier, in human NSCLC models we observed that metabolic targeting with the antidiabetic agent metformin activates the energy stress sensor AMP-activated kinase (AMPK) [8], inhibits the AKT - mammalian target of rapamycin (mTOR) pathway and suppresses tumor growth [9]. On this basis, we conducted a randomized phase II clinical trial, OCOG-ALMERA, to examine whether addition of metformin (2000 mg/day) to standard cCRT could improve survival outcomes in LA-NSCLC. With 54 patients recruited from 7 Canadian centres, the OCOG-ALMERA was not able to demonstrate benefit [10]. Such results illustrate a need for effective biomarkers that could predict patient response to cCRT or investigational targeted therapies.

Growth differentiation factor 15 (GDF15), also known as macrophage inhibitory cytokine 1 (MIC-1) or non-steroidal anti-inflammatory drug-activated gene-1(NAG-1) is a member of the TGFβ superfamily [11, 12]. GDF15 is synthesized initially as a 308aa peptide. After being processed to its mature form, it is released and circulates as a 25 kDa dimer linked by a single interchain disulphide bond [13]. GDF15 mRNA is expressed at a low level in most human tissues except in abdominal and pelvic organs and placenta. In healthy individuals, GDF15 serum content ranges from 200pg/ml to 1200pg/ml [14–16]. Circulating GDF15 is increased in several pathologies, including obesity, diabetes, cardiovascular diseases, and cancer [13]. The only described receptor for GDF15 is GFRAL-RET, expressed specifically in neurons of the area postrema and nucleus of the solitary tract (hindbrain) in mice and humans [17].

GDF15 was shown to help suppress appetite, reduce food intake and body weight [13], and to promote energy expenditure in muscle [18]. We and others found that cytotoxic treatments such as chemotherapy [19] and ionizing radiation [20], or metabolic therapies such as metformin [21–23], induce GDF15 expression. Further, efforts to develop radiation bio-dosimetry tools found that GDF15 belongs to a group of genes exhibiting a linear dose response relationship with radiation dose received in human peripheral blood cells [24].

Studies suggest that GDF15 may have diagnostic and prognostic value in lung cancer [25, 26]. However, the modulation of this marker by cytotoxic therapy and its predictive or prognostic value have not been investigated systematically within lung cancer randomized trials.

Methods

Patient population

With appropriate institutional ethics approvals, OCOG-ALMERA (NCT02115464) accrued 54 patients from 7 Canadian centres. Full details of the trial were described earlier [10]. Briefly, the study accrued non-diabetic patients with pathologically or cytologically proven unresected stage IIIA or IIIB NSCLC (AJCC 7th edition) staged with conventional imaging, whole body fluorodeoxyglucose (FDG) positron emission tomography (PET) and endobronchial ultrasound (EBUS), as clinically indicated. Patients were randomized to either cCRT alone ($n=28$) or combined with metformin ($n=26$).

Interventions

Radiotherapy Chest radiotherapy of 60–66 Gy was delivered in 30 daily fractions over six weeks concurrent with chemotherapy. Radiotherapy volume delineation followed ICRU-62 guidelines [27]. Gross tumor volume (GTV), including the primary tumor and involved lymph nodes only, was expanded to include respiratory tumor motion detected by 4-dimensional CT, fluoroscopy, or estimated (1 to 1.5 cm expansion) for conventional CT simulation, respecting anatomic barriers to define internal target volume (ITV). Planned target volume (PTV) was generated

with the expansion of ITV by 0.5 to 1.5 cm. Normal tissue dose constraints followed standard Radiation Therapy Oncology Group (RTOG) guidelines.

Systemic therapy Platinum-based chemotherapy was given in 2 cycles of 3–4 weeks concurrently with radiotherapy. Acceptable options included cisplatin–etoposide, or cisplatin-vinorelbine combinations. Carboplatin in combination with etoposide or paclitaxel was also permitted. Consolidation chemotherapy of 2 additional cycles was allowed in patients treated with the carboplatin/paclitaxel combination. Anti-programmed cell death receptor ligand 1 (PD-L1) immunotherapy was permitted after May 2018. See Table s1 for details of systemic therapy.

Metformin Treatment started 2 weeks before the initiation of standard therapy at a dose of 1000 mg/d orally; the dose was increased to 1500 mg/d at week 2 and to 2000 mg/d at the start of chemoradiotherapy (week 3 of metformin treatment – week 1 of cCRT). Reduction of the dose escalation period to 1 week was permitted if clinically indicated. Metformin was given concurrently with cytotoxic therapy and as a consolidation treatment at 2000 mg/d daily for up to 12 months unless dose de-escalation was required due to toxicity or disease progression.

Biospecimen collection

Blood specimens were submitted at baseline, 1 week into cCRT treatment (corresponding to the end of the first week of cCRT and the end of the third week of metformin treatment), the day of the last radiotherapy treatment (week 6 of cCRT, or week 8 from initiation of metformin treatment) and at 6 months (26 weeks) from the end of cCRT, while on metformin treatment or observation. EDTA plasma was extracted, preserved at -80ºC and shipped to the biospecimen storage site at Juravinski Cancer Center.

Elisa assay

GDF15 content was analysed by personnel blinded to the outcomes and data collected by the trial. Assays were performed using the GDF15 Duo Set ELISA kit (Catalog #DY957, R&D Systems). Briefly, 96 well plates were coated with the capture antibody for human GDF15. After washing and blocking, 100 µl of standard and samples were added to each well and incubated for two hours, followed by washing, incubation with detection antibody, streptavidin-horse radish peroxidase (HRP) and substrate solution. Assays were terminated with stop solution and optical density was measured with a microplate reader at 450 nm (wavelength correction set to 540 nm).

Statistical analysis

Descriptive statistics were used to summarise patient characteristics, laboratory measures, and outcomes. Kaplan-Meier method was used to estimate overall survival (OS) and relapse-free survival (RFS). Cox regression analyses were used to investigate the prognostic ability of GDF15. In univariable analysis GDF15 levels were stratified by intervention arm. In the multivariable model GDF15, as a continuous variable was adjusted for treatment and AJCC 7th edition overall stage (stage IIIA vs. IIIB). OS and RFS, of all patients, are illustrated vs. baseline GDF15 levels dichotomized at the median marker level, as well as the value of 1465pg/ml, suggested to have significant discriminatory value in other reports of NSCLC patients. An exploratory sensitivity analysis for optimal cutoff values was performed. The change in GDF15 over time was calculated as the absolute difference (=on treatment - baseline) and relative change (= (on treatment – baseline)/baseline*100%) and tested using the Wilcoxon rank sum test. Spearman correlation coefficients were used to explore the association between GDF15 and radiotherapy treatment volumes. Confidence intervals were constructed for selected outcomes of interest. All tests and confidence intervals were two-sided, and statistical significance was defined at the $\alpha=0.05$ level. All analyses were done blinded to patient outcomes.

Results

Population

Of the 54 patients accrued to the OCOG-ALMERA trial, 32 patients [16 of the control (cCRT) and 16 of the experimental arm (cCRT + metformin)] provided blood specimens to extract EDTA plasma at baseline and at least one of the subsequent time-points and were included in this analysis. There were no statistically significant differences in patient characteristics between treatment arms amongst the patients participating in this analysis (Table 1). Further, there were no statistically significant differences in patient characteristics between trial patients with available biospecimens included in this analysis vs. those that were not (Table s1).

Baseline GDF15 levels and kinetics during chemoradiotherapy

GDF15 levels detected at baseline, were generally elevated, median = 1030, (range = 417-2445pg/ml), compared to reported normal plasma levels [26, 28] (Fig. 1A). There was no statistically significant difference between GDF15 levels measured at baseline between the cCRT and the cCRT + metformin arms (Fig. 1B, Table s2).

After only 1 week of cCRT, GDF15 levels increased, remained elevated until the end of cCRT (week 6), and returned to approximate pre-treatment levels at 6

Table 1. Patient characteristics. Clinical, disease stage, histological and treatment characteristics of the OCOG-ALMERA patients included in this analysis

		CRT alone (standard)	CRT + Metformin (experimental)	*p*-values
N		16	16	
Stage	N (%) 3B	8 (50.0)	6 (37.5)	0.72
Stratum Treatment	N (%) Carboplatin	2 (12.5)	0	0.34
	N (%) Cisplatin	4 (25.0)	4 (25.0)	
	No stratum listed	10 (62.5)	12 (75.0)	
Sex	N (%) Female	8 (50.0)	10 (62.5)	0.72
ECOG	0	7 (43.8)	10 (62.5)	0.48
	1	9 (56.3)	6 (37.5)	
Age	Mean (SD)	64.4 (8.5)	65.0 (5.6)	1.00
BMI	Mean (SD)	25.6 (4.5)	26.5 (5.4)	0.65
Histology	SCC	8 (50.0)	4 (25.0)	0.25
	Adenocarcinoma	8 (50.0)	11 (68.8)	
	NOS	0	1 (6.3)	
Chemotherapy cycles	1	0	2 (12.5)	0.33
	2	10 (62.5)	8 (50.0)	
	Missing	6 (37.5)	6 (37.5)	
Duration of Chemotherapy	Median (Range) days	35 (28, 44)	35 (4, 42)	0.094
	N (%) > = 35 days	14 (87.5)	10 (62.5)	0.22
Immunotherapy	N (%) Yes	4 (25.0)	2 (12.5)	0.65
Radiotherapy Dose	< 60 Gy	0	2 (12.5)	0.32
	60 Gy	8 (50.0)	8 (50.0)	
	> 60 Gy	8 (50.0)	6 (37.5)	
GTV	Median (IQR)	113 (64, 172)	124 (46, 166)	0.84
ITV	Median (IQR)	156 (95, 323)	154 (52, 249)	0.74
PTV	Median (IQR)	437 (309, 540)	411 (252, 540)	0.60

months after completion of cCRT (Fig. 2A). Interestingly, the addition of metformin to cCRT, in the experimental arm was associated with a greater increase in GDF15 levels during cCRT, compared to cCRT alone (Fig. 2B, Table s2).

Plasma GDF15 levels correlate with radiotherapy tumor volumes

Given the possible modulation of GDF15 by cCRT we analysed the relationship between the levels of this marker and radiotherapy tumor volumes: gross tumor volume (GTV) and planned tumor volume (PTV) (median and range values are shown in Table 1). Using Spearman correlation coefficient, we detected positive, moderately significant, correlations between GDF15 and GTV at baseline ($p<0.01$), and between GDF15 and PTV at 1 week into cCRT ($p<0.006$) (Fig. 3).

We did not detect any obvious difference in baseline GDF15 values when patients were separated per AJCC 7th edition staging factors, including overall stage (IIIA vs. IIIB), T stage (T1-2 vs. T3), and N stage (N0-1 vs. N2-3) (Table s3).

Prognostic analysis of baseline GDF15 levels

Kaplan-Meier survival curves illustrating the overall survival (OS) and the relapse free survival (RFS) of patients included in the analysis are shown in Fig. 4 and Fig. s1. A statistically significant inverse association was observed between baseline GDF15 levels with OS and RFS. In univariate analysis, T- and N- stage score, but not overall stage or tumor volumes (GTV, PTV), could predict RFS (Table 2). However, only baseline GDF15, was found to be significantly prognostic for both RFS [HR 1.11 (95% CI: 1.02, 1.22), $p=0.016$] and OS [HR 1.17 (95% CI: 1.04, 1.32), $p=0.009$]. Further, baseline plasma GDF15 was

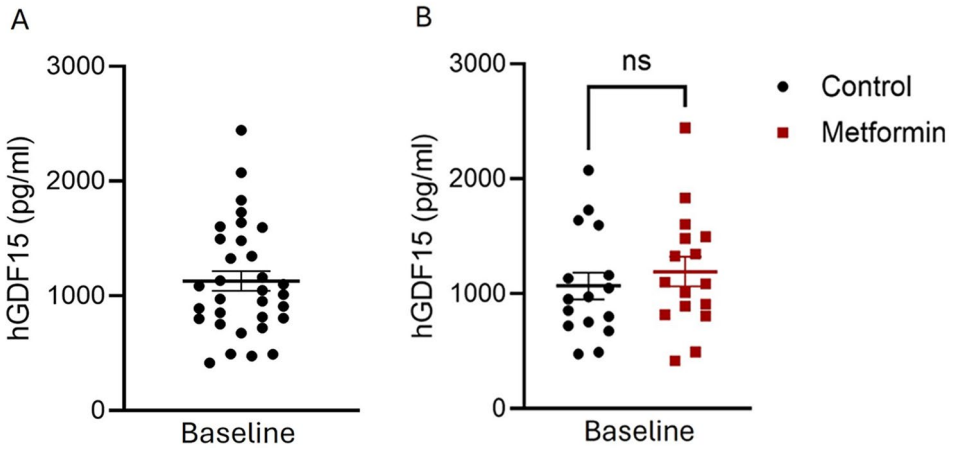

Fig. 1 Distribution of baseline plasma GDF15 levels. **(A)** Distribution of individual baseline GDF15 levels for all patients. **(B)** Comparison of baseline GDF15 levels between the concurrent chemo-radiotherapy (cCRT) and the cCRT + metformin arms (Wilcoxon rank sum test $p=0.44$: [median = 963.5pg/ml (475.98-2072.85pg/ml) vs. 1094.2pg/ml (417.23-2444.59pg/ml), respectively]

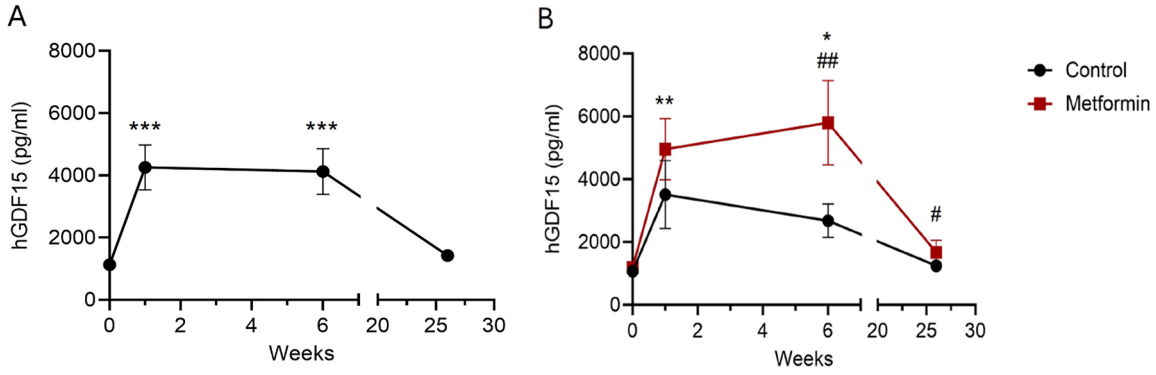

Fig. 2 Modulation of plasma GDF15 levels by treatments. Levels of GDF15 over the first 26 weeks (6 months) from initiation of cCRT (assessments on weeks 1, 6 and 26 of cCRT or weeks 3, 8 and 26 of metformin treatment). **(A)** For all patients included in this analysis. **(B)** For patients based on treatment arm (cCRT: Control, cCRT + Metformin: Metformin). Statistical significance compared to average baseline plasma GDF15 levels, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Statistical significance compared to average plasma GDF15 level of the Control (cCRT) arm patients at the same timepoint: # $p < 0.05$, ## $p < 0.01$

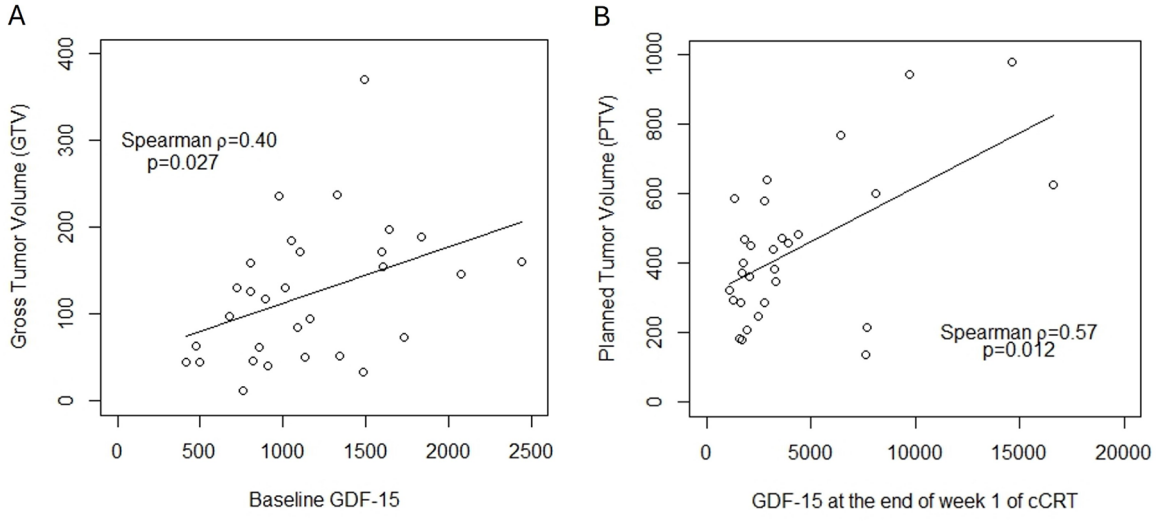

Fig. 3 Association of plasma GDF15 with tumor volumes. **(A)** Correlation of baseline GDF15 levels with gross tumor volume (GTV). **(B)** Correlation of circulating GDF15 levels at the end of week 1 of concurrent chemoradiotherapy (cCRT) with planned tumor volume (PTV).

prognostic for poor relapse-free (RFS) and overall survival (OS) in multivariate analysis ($p=0.005$ and $p=0.002$, respectively).

Patients with baseline GDF15 above the median (1029.9pg/ml) showed reduced OS **(A)** compared to the those below the median [HR=1.19, (95% CI:1.06,1.34), $p=0.005$] and similarly reduced RFS **(B)** [(HR=1.14 (95% CI:1.04, 1.27), $p=0.005$]. An exploratory sensitivity analysis suggested that GDF15 levels between 1000pg/ml – 1700pg/ml was the range of values which discriminated between better and worse OS and RFS (see Table s4). Indeed, the GDF15 baseline cut-off value of 1465pg/ml reported by Liu et al. [28] dichotomized our patients also in two groups with significantly different OS (HR=5.29, (95% CI:1.46, 19.15), $p=0.011$) (Fig. 4C).

Although, cCRT and metformin treatments were associated with increases in GDF15 levels during treatment (Tables s2A/B), we did not observe clear trends suggesting that changes in GDF15 could be prognostic for outcomes. However, the available power for this analysis was limited and affected by multiplicity testing.

Discussion

The key observation of this study is that baseline plasma GDF15 may be an independent prognostic biomarker in unresected LA-NSCLC patients managed with standard of care cCRT. Interestingly, in this analysis GDF15 was found to have improved prognostic value compared to AJCC tumor stage and tumor volumes defined for radiotherapy treatment.

Earlier reports with lung cancer patients suggested the biomarker value of GDF15. Liu et al. (2016) [28] detected prognostic value for GDF15 in stage I and II NSCLC patients managed with surgery, and others reported similar observations in undifferentiated populations including all stages of NSCLC [29]. All studies reported

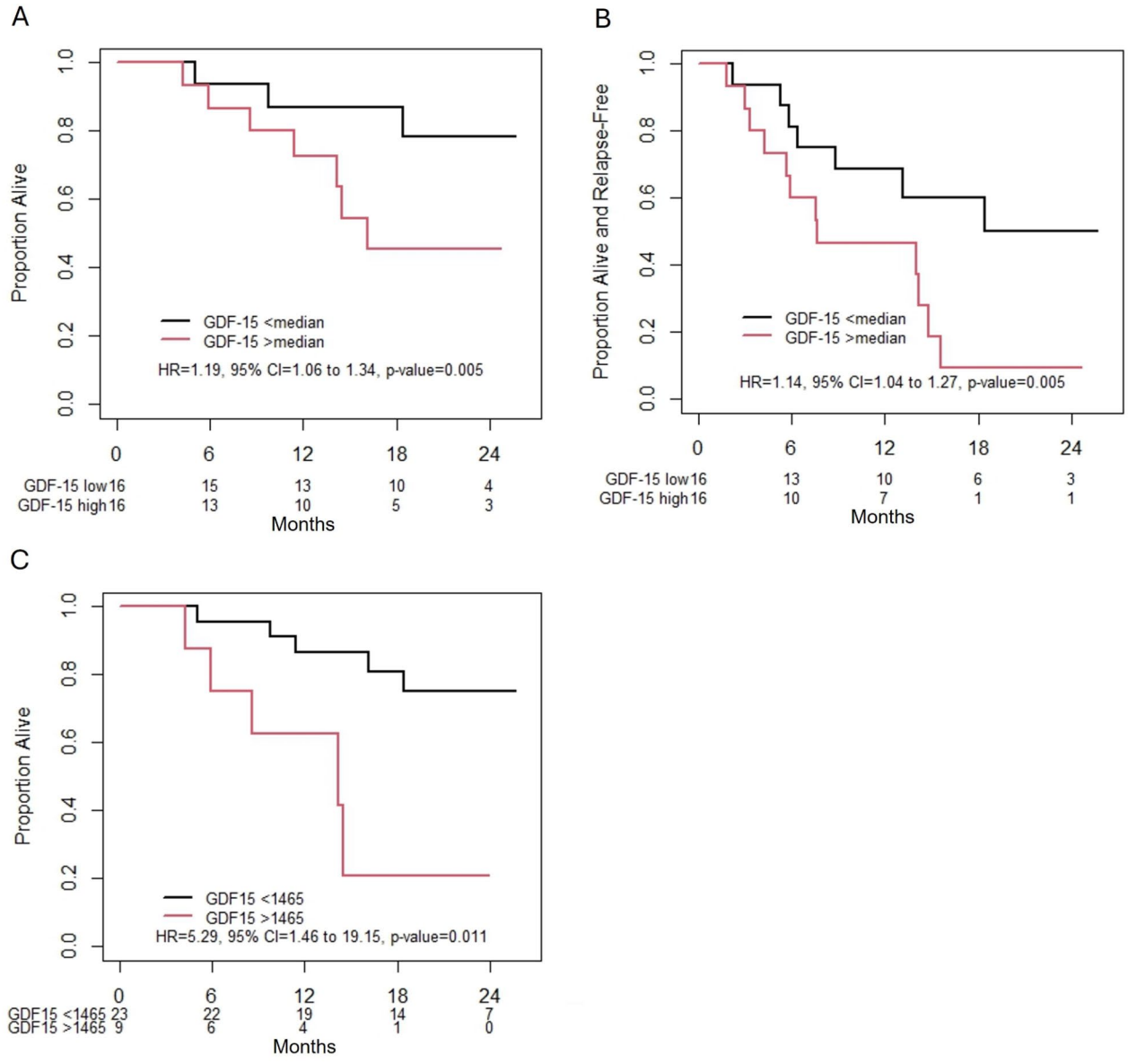

Fig. 4 Kaplan-Meier survival curves of overall (OS) and relapse free (RFS) survival in relation to baseline GDF15 levels for the entire population. **A**. OS and **B**. RFS of patients based on baseline GDF15 levels (black line: below the median of the entire population; red dotted line: above the median). **C**. Overall survival (OS) of patients based on baseline GDF15 levels using the cutoff value of 1465pg/ml reported by Liu et al. (2016)

elevated baseline GDF15 levels in lung cancer patients. Consistently, in the present study we detected elevated levels of GDF15 in stage III NSCLC patients at baseline. Our sensitivity analysis of cutoff values indicated that GDF15 levels > 1000pg/ml can predict poor survival outcomes in unresected LA-NSCLC treated with cCRT. Indeed, the median of our population (1029.9pg/ml) separated patients into two groups with significantly different overall survival (OS) independently of the treatment arm distribution ($p=0.005$), and when adjusted for treatment arm ($p=0.0009$). Further, our data validated the cutoff level of 1465pg/ml described by Liu et al. (2016) [28] to predict poor post-operative outcomes. GDF15 levels > 1000 pg/ml deserve further systematic investigation as a potentially poor prognosis marker for NSCLC patients.

Given that elevated baseline GDF15 predicted poor RFS, it is possible that GDF15 may be associated with factors that determine the biological response of lung cancer to cCRT. Pre-clinical studies suggested that GDF15 could mediate radio-resistance through a variety of mechanisms, including promotion of endothelial to mesenchymal transition (EMT), regulation of reactive oxygen species, inhibition of apoptosis and induction of

Stage Factor (AJCC 7th Edition) Tumor volumes / plasma biomarker	RFS		OS	
	HR (CI)	p-value	HR (CI)	p-value
Overall stage (IIIA vs IIIB)	1.20 (0.47, 3.09)	0.7	1.24 (0.34, 4.53)	0.75
T stage (1-2 vs 3-4)	1.45 (1.01, 2.08)	0.044	1.14 (0.75, 1.75)	0.54
N stage (0-1 vs 2-3)	0.52 (0.26, 1.06)	0.072	0.47 (0.19, 1.17)	0.11
GTV	1.00 (1.00, 1.01)	0.47	1.00 (0.99, 1.01)	0.43
PTV	1.00 (1.00, 1.00)	0.47	1.00 (0.99, 1.00)	0.16
GDF15	1.11 (1.02, 1.22)	0.016	1.17 (1.04, 1.32)	0.009

Table 2 Prognostic value of baseline GDF15 compared to tumor volume and stage factors (*Univariate analysis*). Hazard Ratio (HR), confidence interval (95% CI) and p-values are illustrated for relapse free (RFS) and overall (OS) survival

cell cycle arrest [20, 30, 31]. On the other hand, reports suggested that the pre-treatment tumor volume determines response to cytotoxic therapy [32, 33]. In this analysis we could not detect an association of tumor volumes (GTV or PTV) with RFS but found a moderately significant association of baseline GDF15 with GTV. This indicates that GDF15 may be able to reflect the disease burden in LA-NSCLC.

Currently, there is need for markers to monitor biological tumor response during cytotoxic therapy. It is possible that GDF15 could serve this role for NSCLC tumors during cCRT. To date, only few studies examined the modulation of GDF15 during cytotoxic treatment. Frey et al. [34] described increased GDF15 in response to either intensity-modulated or stereotactic ablative radiotherapy treatment of prostate cancer. Consistent with this and other observation of GDF15 induction by chemotherapy and radiation [20, 26, 30, 35, 36], we found a significant increase in GDF15 levels measured at the end of weeks 1 and 6 of cCRT (Fig. 2). Remarkably, circulating GDF15 levels 1 week into cCRT correlated with PTV, indicating a potential relationship of this marker with volumes of irradiated tissues in the chest. The increase of GDF15 during cCRT observed in the present study was several-fold above baseline. However, at 6 months after completion of cCRT, GDF15 returned to values close to baseline. This is not dissimilar to other reports [26, 34].

Studies suggested that GDF15 may contribute to the anti-hyperglycaemic effect of metformin [13, 22]. In 2317 dysglycemic patients that were part of the Outcome Reduction with Initial Glargine Intervention (ORIGIN) trial, GDF15 circulating levels were strongly linked to metformin use [21]. Similar results were reported by Coll and colleagues in 2020 amongst the participants of CAMERA, a randomized placebo-control trial testing metformin in people without diabetes but with a history of cardiovascular disease [37]. Metformin treatment was significantly associated with increased levels of circulating GDF15 at 6, 12, and 18 months.

Here, we observed a greater increase of plasma GDF15 levels in LA-NSCLC patients who received metformin treatment in addition to cCRT. Given the recent evidence generated by our group and others [38, 39] that (i) GDF15 mediates energy expenditure [18], (ii) is responsible for the chemotherapy-induced anorexia and weight loss [19] and (iii) is associated with cachexia observed in lung cancer patients [38], it is possible that GDF15 could influence survival outcomes in patients of poor performance status when metabolic targeting is added to cytotoxic therapy. This notion requires careful investigation. Early industry trials investigate GDF15-targeting antibody therapies as anti-cancer or anti-cachexia agents, alone or in combination with standard therapies (NCT04725474, NCT05397171, NCT05546476).

This is the first study that investigated the prognostic value of GDF15 as a biomarker for tumor response to cCRT and overall survival within a prospective randomized clinical trial in LA-NSCLC. The use of a consistent methodology in biomarker analysis and strict guidelines for patient selection, specimen collection and handling paired with consistent tumor delineation for radiotherapy planning within this trial may have contributed to the ability of this analysis to detect prognostic biomarker value of GDF15.

Limitations of this analysis are the small sample size, the open-label nature of the OCOG-ALMERA trial and the heterogeneity of our population. It is possible that GDF15 modulation in this population may be affected by other interfering factors. The moderately significant associations between GDF15 levels and tumor volumes detected in this study are interesting but should be interpreted with caution. Further, the number of patients with samples at later timepoints was limited, and it is possible that observed results are biased by including only those

with available data. Therefore, it is necessary to validate the results of our study in larger trials and datasets.

Conclusions

The results obtained in this study give insights into the potential role of GDF15 as a marker of prognosis and tumor response to cCRT in LA-NSCLC. GDF15 may be able to function also as a useful marker of systemic response to metabolic therapy in lung cancer patients. Our results warrant further validation in biospecimens of larger lung cancer randomized trials.

Abbreviations

GDF15	Growth differentiation factor 15
TGFβ	Transforming growth factor beta
MIC-1	Macrophage inhibitory cytokine 1
NAG-1	Non-steroidal anti-inflammatory drug-activated gene-1
LA-NSCLC	Locally advanced non-small cell lung cancer
cCRT	Concurrent chemoradiotherapy
AMPK	AMP-activated kinase
AJCC	American Joint Committee on Cancer
FDG	18-fluoro-deoxyglucose
PET	Positron emission tomography
EBUS	Endobronchial ultrasound
GTV	Gross tumor volume
PTV	Planned target volume
ITV	Internal target volume
PD-L1	Programmed cell death receptor ligand 1

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13014-024-02546-y.

Supplementary Material 1

Author contributions

FDP: Investigation, Visualization, Data Curation, Writing - Original Draft. GP: Formal analysis, Data Curation. EET: Visualization, Writing - Original Draft, Data Curation. AG: Writing - Original Draft. EA: Investigation. ODB: Investigation. AA: Investigation. AS: Investigation. GO: Investigation. PME: Investigation. BA: Investigation. NA: Investigation. AR: Investigation. WR: Investigation. MV: Investigation. PK: Methodology. MW: Methodology. JW: Investigation, Methodology. GS: Methodology, Resources, Writing - Original Draft, Supervision. TT: Conceptualization, Methodology, Investigation, Resources, Writing - Original Draft, Supervision, Project administration, Funding acquisition.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethical approval

The clinical trial protocol of OCOG-ALMERA including the section of biospecimen collection and corresponding consent forms, received Health Ethics approval from all institutional and provincial ethics boards of participating institutions. This included the Ontario Cancer Research Ethics Board (OCREB), McGill University Research Ethics Board (REB), University of Manitoba REB and Health Research Ethics Board of Alberta (HREBA). Laboratory analysis was conducted with approval by the Hamilton integrated Research Ethics Board (HiREB) (#17511).

Consent to participate

All patients willing to provide biospecimen signed the ethics board approved informed-consent forms before donating their biospecimen. This consent form clearly stated that biospecimen will be collected, shipped to Juravinski Cancer Centre, Hamilton, Ontario, and will be used for the purpose of biomarker analysis, in combination with clinical data, in an anonymized fashion.

Competing interests

The authors declare no competing interests.

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