Poster 5381

Proteomic analysis to study selumetinib effect in adolescents with Neurofibromatosis Type-1 and inoperable plexiform neurofibroma

Haydee Lara PhD,[1, 2] and Dhirender Ahlawat,[1]

[1]Alexion-AstraZeneca Rare Disease Unit, Translational Sciences
[2]Corresponding Author

OBJECTIVES

To perform proteomic analysis in samples from study NCT05101148, and to investigate the effect of selumetinib in disease-relevant pathways in NF1 patients, especially in patients with skeletal manifestations.

CONCLUSIONS

This proteomics study in adolescent NF1 patients treated with selumetinib, demonstrates changes in proteins previously reported for this population, such as CCL5 (RANTES). Some proteins associated with tumor growth were downregulated. The analysis also uncovered a potential link of collagen proteins dysregulation in NF1 skeletal manifestations with other bone diseases.

PLAIN LANGUAGE SUMMARY

Why did we perform this research?
We studied changes in blood proteins in NF1 patients that received selumetinib to understand the impact of treatment in known disease pathways and uncover new pathways with potential for treatment.

How did we perform this research?
We analyzed 11,000 proteins in blood from 23 adolescent NF1 patients treated with selumetinib.

What were the findings of this research and what are the implications?
We found dysregulation of proteins related to tumor growth in NF1 patients treated with selumetinib. Additionally, collagen proteins were dysregulated in NF1 patients with skeletal manifestations. Further studies are necessary to better understand the potential of these proteins as targets for treatment.

This study was funded by the NF1 Global Project Team at Alexion-AstraZeneca Rare Disease Unit (RDU).

Acknowledgements:

The Koselugo Global Project Team: Sergii Derkach, Idoia Herrero, Mel Cornford, Ruth Viana-Alvarez*, Karen So*, Eric Fan*, Gail Doughton, Heeyung Parsons*

**Past members of the Koselugo Global Project Team*

Alexion RDU, Translational Sciences: Ruba Bou-Chahine, Meera Ramanujan, Russ Weiner

Alexion RDU, Operations & Outsourcing: Marianne Flynn, Radoslaw Knura, Walter Espinoza

We thank the patients who participated in this study, their families, and the study investigator teams.

Disclosures: H. Lara and D. Ahlawat are AstraZeneca employees.

Poster presented at the 2025 Children's Tumor Foundation's annual NF Conference by Haydee Lara, PhD.
Corresponding author email address: Haydee.Lara@alexion.com, Haydee.Lara@astrazeneca.com

BACKGROUND & PURPOSE

The samples analyzed in this proteomics study correspond to clinical study NCT05101148 "Phase I Study to Assess the Effect of Food on the PK and Gastrointestinal Tolerability of Selumetinib in Adolescent children With Neurofibromatosis Type 1 Related Plexiform Neurofibromas". The clinical study NCT05101148 was designed to evaluate the effect of a low-fat meal on steady state selumetinib exposure. Selumetinib was approved in the US in 2020 and in Europe in 2021; it was approved for the treatment of Neurofibromatosis Type-1 (NF1) pediatric patients with symptomatic and inoperable Plexiform Neurofibroma (PN) and until recently, the drug had to be taken in a fasted state. The results of this clinical study supported the update of the label in 2024, and selumetinib can now be taken with or without food. In the proteomic analysis discussed here, we investigated the effect of selumetinib in disease-relevant pathways in this NF1 patient population, especially in the patients with skeletal manifestations.

METHODS

- We evaluated 11,000 proteins in blood plasma samples corresponding to 23 patients from clinical study NCT05101148 The analysis included pre-dose blood plasma collected during Cycle 1 (T1) and Cycle 2 (T2) of selumetinib treatment
- Proteomics blood measurements were targeted for Day 8 but could be taken on any day between Days 4 and 14, assuming the participant received at least one consecutive day of dosing before this sample was taken
- The samples were analyzed using aptamer-based proteomics technology
- We compared protein fold changes in:
  - Pre-dose plasma samples corresponding to Cycle 1 vs Cycle 2 of selumetinib treatment
  - Patients with skeletal manifestations vs patients without skeletal manifestations
- We also performed pathway enrichment analysis

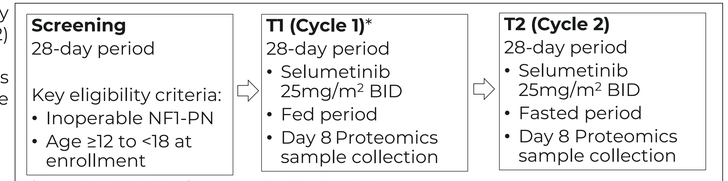

Figure 1. Study Design of NCT05101148. *Participants took selumetinib with a low-fat meal for 28 days (Cycle 1 or T1), followed by a 7-day washout period. Then selumetinib was administered again for another 28 days (Cycle 2 or T2).*

RESULTS AND INTERPRETATION

Proteomic Data Analysis.

We performed proteomic data analysis using the company proprietary software (Somalogic Data Delve Statistics):

- Test Type: t-test:
  - Cycle 1/T1 vs Cycle 2/T2
  - Non-skeletal vs skeletal
- Criteria: Fold change, log2 = ± 0.6 (~1.5 fold change); p-value ≤0.05
- In these assays, protein expression is measured in Relative Fluorescence Units (RFU)

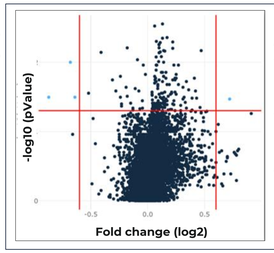

Figure 2. Analysis of proteomic profiles of Cycle 1 vs Cycle 2 of selumetinib treatment. *In this analysis,* ***CCL5 (RANTES) is upregulated*** *in NF1 patients after 2 cycles of selumetinib treatment (blue dot in the upper right quadrant of volcano plot).* ***Three proteins were downregulated: Hepcidin (HAMP), Carnosine (CNDP1) and HMG Co-A synthase*** *(HMGCS1). 3 proteomic datapoints were excluded due QC flags.*

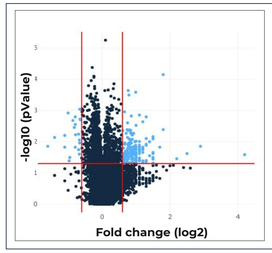

Figure 3. Analysis of non-skeletal vs skeletal groups. *In study NCT05101148, 10 of 23 patients presented skeletal manifestations. We excluded 3 proteomic datapoints from this t-test analysis after data QC, therefore this analysis only includes 8/20 patients with skeletal manifestations.* ***152 proteins are upregulated in NF1 patients with skeletal manifestations, and 22 are downregulated.***

Proteins associated with tumor growth are regulated in NF1 patients treated with selumetinib.

We found that CCL5 (RANTES) was increased in NF1 patient samples during Cycle 2 of selumetinib treatment, while proteins associated with tumor growth, like hepcidin and HMG Co-A synthase, were decreased.

- CCL5, expressed by immune cells, can have antitumor and immunosuppressive effects and is linked to NF1 morbidity. It is upregulated in NF1-related tumors such as MPNST and OPG.
- HAMP has no known role in NF1, but its inhibition may hinder cancer growth.
- CNDP1 has no established NF1 role but is dysregulated in other tumors.
- HMGCS1 is upregulated in many tumors and statins targeting it improve NF1 cognitive deficits.

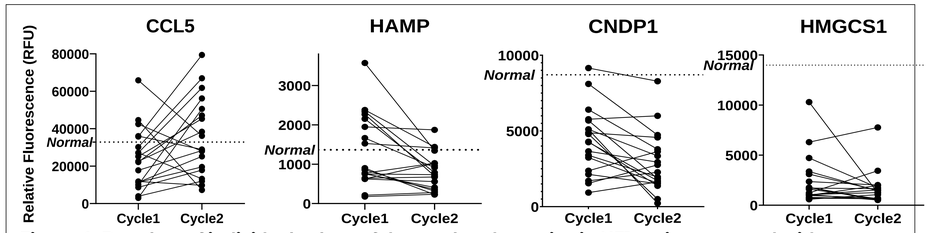

Figure 4. Dot plots of individual values of dysregulated proteins in NF1 patients treated with selumetinib. Only patients for which both datapoints, Cycle 1 and Cycle 2 are represented. 3 samples were excluded after data QC. Normal RFU value is not age matched.

Collagen proteins are dysregulated in NF1 patients with skeletal manifestations.

The t-test of non-skeletal vs skeletal datapoints demonstrated that 152 proteins were upregulated in NF1 patients with skeletal manifestations, and 22 were downregulated. The Table below shows the Top 15 upregulated and downregulated proteins, sorted by fold change.

Downregulated proteins

Protein Name	Gene Symbol	Fold Change	p- value
T-cell surface antigen CD2	CD2	-1.6	0.014
Probable glutamate--tRNA ligase; mitochondrial	EARS2	-1.4	0.0073
Leptin	LEP	-1.1	0.0063
Leptin	LEP	-1.1	0.015
Leukocyte immunoglobulin-like receptor subfamily A member 6	LILRA6	-1	0.0012
Mitogen-activated protein kinase kinase kinase kinase 1	MAP4K1	-0.97	0.041
Thyroglobulin	TG	-0.95	0.032
Proline-rich protein 23C	PRR23C	-0.86	0.0021
Growth hormone variant	GH2	-0.82	0.0035
I-kappa-B-epsilon	NFKBIE	-0.79	0.015
VPS10 domain-containing receptor SorCS2	SORCS2	-0.79	0.0028
Protocadherin-10:Cytoplasmic domain	PCDH10	-0.79	0.0016
Liver-expressed antimicrobial peptide 2	LEAP2	-0.78	0.006
DNA-directed RNA polymerase I subunit RPA34	POLR1G	-0.69	0.018
Neurotensin/neuromedin N	NTS	-0.69	0.0057

Upregulated proteins

Protein Name	Gene Symbol	Fold Change	p-value
Histo-blood group ABO system transferase	ABO	4.2	0.026
Chondrocalcin	**COL2A1**	**2.9**	**0.014**
Collagen alpha-1(IX) chain	**COL9A1**	**2.5**	**0.024**
Collagen alpha-1(X) chain	**COL10A1**	**2.2**	**0.035**
Rho family-interacting cell polarization regulator 2	RIPOR2	1.8	0.000071
Heterogeneous nuclear ribonucleoprotein A0	HNRNPA0	1.8	0.0041
Serine/threonine-protein kinase PAK 6	PAK6	1.7	0.015
Ubiquinone biosynthesis protein COQ9; mitochondrial	COQ9	1.5	0.0069
Transcription factor SOX-4	SOX4	1.5	0.0081
Stathmin-4	STMN4	1.5	0.022
CUGBP Elav-like family member 3	CELF3	1.5	0.024
Low affinity immunoglobulin gamma Fc region receptor II-b	FCGR2B	1.5	0.032
Collagen alpha-2(XI) chain	**COL11A2**	**1.5**	**0.036**
Matrilin-3	MATN3	1.5	0.049
Friend leukemia integration 1 transcription factor	FLI1	1.4	0.01

Proteins KEGG Pathway Analysis of upregulated proteins in NF1 skeletal patients.

Base Excision Repair and Hypoxia Inducible Factor pathways were regulated (with an input of 152 upregulated proteins in g:Progiler software) :

- BER is a DNA damage repair pathway for the processing of small base lesions; one study showed deficient DNA damage repair in NF1 patients (Gutierrez *et al.*) and there is one case report of co-occurrence of NF1 with a DNA repair disease (Guerrini-Rousseau *et al.*)
- HIF-1 is a transcription factor that regulates oxygen homeostasis
- In solid tumors, HIF-1 promotes cell survival and migration, stimulates angiogenesis, and induces resistance to radiation and chemotherapy
- Hypoxia Inducible Factors have a role in neurofibromin (NF1) degradation and activation of MERK/ERK (Green *et al.*)

Pathway	Adjusted p-value	Intersections
Base excision repair (BER)	0.0043	PNKP,PCNA,HMGB1,PARP1,APEX1
Signaling pathway (HIF-1)	0.0430	ENO2,ENO1,HK2,CAMK2B,CAMK2D, PRKCB

References:

1. clinicaltrials.gov/study/NCT05101148
2. Viskochil *et al.*, 2024. Effect of food in selumetinib pharmacokinetics...in adolescents with neurofibromatosis type-1...
3. Gutierrez et al., 2014. DNA damage and repair capacity in patients with neurofibromatosis type 1.
4. Guerrini-Rousseau et al., 2024. Neurofibromatosis type 1 mosaicism in patients with constitutional mismatch repair deficiency.