IL-1RAP Is a Targetable Surface Antigen in Relapsed/Refractoy AML

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INTRODUCTION

Relapsed/Refractory (R/R) AML is an unmet medical need. Novel AML immunotherapies require surface antigens that have broad expression, both at the inter-individual level and intra-individual (blasts and leukemic stem cells [LSCs]) levels, with minimal expression on healthy HSPCs.

IL-1RAP, the accessory co-receptor of the interleukin 1, 32, and 33 receptors, is a promising target antigen with broad expression on AML cells at diagnosis (Boissel et al. EHA 2024 #P453, De Dreuzy et al. AACR 2024 #6322) and has been suggested as a potential Leukemic Stem Cell (LSC) marker (Askmyr et al. Blood 2013). Its expression in R/R AMLs eligible for early-stage trials is unknown.

AIM

The French ALFA group initiated a prospective 2-cohort (newly-diagnosed, relapsed/refractory) observational study in 2022. The ALFA-PPP study (NCT04777916) aims to prospectively collect clinical data and bio-samples in all AML patients aged 18 years old or more referred to 27 ALFA centers.

We aimed to determine IL-1RAP expression on blasts and LSCs in R/R AMLs prospectively accrued in the ALFA-PPP study.

METHODS

Peripheral Blood (PB) or Bone marrow (BM) from relapsed/refractory (excluding molecular relapse) AML patients ≥18 years old accrued to the ALFA PPP registry (NCT04777916) were collected and prospectively assessed for IL-1RAP expression (clone B-R58) in a 10-marker panel on an Ique3 high-throughput cytometer (Sartorius) and analysed on Kaluza (Beckman).
LSCs are defined as CD34+/CD38-/(CD97|TIM3|CLL1)+ cells.
Clinical annotations were derived from the ALFA-PPP eCRF (Dombret et al. ASH 2024 #2424). Centralized genomics (37-gene panel) was done in the ALFA Central Genomics Lab (Lille University Hospital, France).

IL1RAP	CD97	TIM3	CLL1	CD45
CD45RA	GPR56	CD33	CD34	CD38

RESULTS

First 38 patients included in the study

1) DEMOGRAPHY

Clinical characteristics

Median age, years (range)	67y (29 - 88)
Gender M/F, N (%)	19/19 (50/50%)
Median BM blast percentage (IQR)	39% (6- 99)
AML type, N (%)	
• *de novo*	21 (58%)
• post-MDS	6 (17%)
• Therapy-related	9 (25%)
Disease Stage, N (%)	
• 1st relapse	14 (37%)
• 2nd relapse or more	9 (24%)
• Relapse not otherwise specified	5 (13%)
• Primary refractory	10 (26)
Prior intensive chemotherapy, N (%)	
• Yes	23 (79%)
• No	6 (21%)
Prior venetoclax exposure, N (%)	
• Yes	14 (58%)
• No	10 (42%)
Prior allogeneic HCT, N (%)	
• Yes	5 (21%)
• No	19 (79%)

Biological characteristics

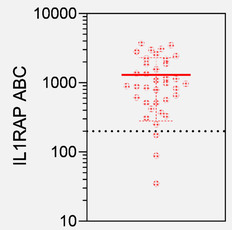
***NPM1* status,** N (%)	
• Mutated	6 (25%)
• Wild type	18 (25%)
FLT3-ITD, N (%)	
• ***Present***	7 (29%)
• Absent	17 (70%)
***IDH 1/2* mutations**, N (%)	
• *IDH1* or *IDH2* mutated	4 (17%)
• *IDH1* and *IDH2* wildtype	20 (83%)

Specimen characteristics

Processing method, N (%)	
• Thawed	6 (16%)
• Fresh	32 (84%)
Sample source, N (%)	
• Bonne marrow	17 (45%)
• Blood	21 (55%)

2) IL-1RAP expression

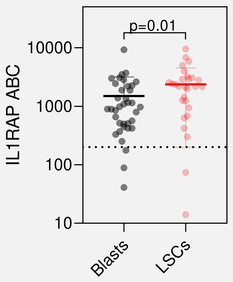
Figure 1. IL-1RAP expression in R/R AMLs



On a median of 20,508 gated blasts (IQR 7,796-31,858), the median proportion of IL-1RAP expressing cells was 24% (IQR 11-55, range 1-99) and the median Antibody Binding Capacity (ABC) was 970 (IQR 514-1,892, range 35- 3,722).

Based on IL-1RAP ABC distribution in CD45high/SSClow lymphocytes used as internal controls (median 38, IQR 16-96), we defined ABC ≥200 as the definition of IL-1RAP positivity in blasts; 34 (89%) patients had IL-1RAP+ blasts.

Figure 2. IL1RAP expression on blasts and LSCs



A median of 2560 (IQR 542-9,960) CD34+/CD38-/ (CD97|TIM3|CLL1)+ LSCs were acquired. In the 29 pts with at least 200 gated LSCs, median IL-1RAP ABC in LSCs was 2,176 (IQR 1,231-3,059), representing a strong enrichment compared to the total blast population (p=0.006).

3) Clinical and biological predictors

Variables, Median (IQR)	IL1RAP - expressing cells (%)	p-value	IL1RAP RFI	p-value	IL1RAP ABC	P value
Sex						
Male	27 (11 - 34)	0.80	2.2 (1.8 – 4.7)	0.92	893 (659 - 1462)	0.64
Female	24 (12 - 37)		2.5 (1.7 – 5.1)		1410 (468 - 2348)	
Age, years	0.18	0.20	0.09	0.59	0.21	0.21
AML ontogeny						
Therapy-related	16 (11 - 37)		2.4 (1.6 – 4.1)		1026 (408 - 1689)	
Post MDS	26 (9 - 48)	0.69	2.1 (1.4 – 4.1)	0.31	902 (275 - 1098)	0.68
De novo	30 (12 – 56)		2.7 (1.7 – 6)		987 (614 – 2189)	
***NPM1* status**						
Mutated	55 (28 - 81)	**0.02**	5.6 (4.2 – 6.6)	**0.03**	1724 (1426 – 1917)	**0.04**
Wild type	14 (5 - 24)		2.1 (1.6 – 2.7)		1016 (370 – 1140)	
***FLT3-ITD* status**						
Present	56 (32 - 77)	**0.01**	6 (4.5 - 6.7)	**0.001**	1878 (1474 – 2315)	**0.004**
Absent	12 (5 - 24)		2.1 (1.6 – 2.3)		913 (360 – 959)	
***IDH1/2* status**						
Mutated	40 (20 - 67)	0.31	4.1 (1.5 – 12.7)	0.78	1042 (430 - 1725)	0.91
Wild type	20 (7 - 47)		2.2 (1.9 – 4.5)		898 (608 - 1628)	
Disease Stage						
First Relapse	31 (14 - 56)		2.7 (2.1 – 5.2)		1155 (856 – 2189)	
2d or + Relapse	24 (5 – 40)	0.48	2.1 (1.6 – 3.8)	0.56	888 (421 – 1378)	0.45
Relapse (not otherwise specified)	52 (15 – 82)		2.3 (2 - 5.3)		1357 (658 – 3093)	
Primary Refractory	20 (10 – 30)		2 (1.6 – 2.7)		724 (405 – 1064)	
Previous Therapies						
Intensive						
Chemotherapy						
Yes	24 (9 - 56)	0.95	2.1 (1.6 – 5.3)	0.83	1254 (468 – 2033)	0.72
No	20 (19 - 21)		2.5 (2.1 – 2.7)		987 (834 - 1155)	
Venetoclax						
Yes	21 (9 - 52)	1	2.3 (2 – 2.8)	0.67	987 (514 – 1357)	0.74
No	19 (9 - 70)		2.1 (1.6 – 5.2)		877 (514 – 2512)	
Allo-HCT						
Yes	5 (4 – 55)	0.31	2 (1.7 – 5.2)	0.60	794 (330 – 2254)	0.64
No	22 (12 – 55)		2.3 (1.7 – 5.4)		943 (594 – 1801)	

There was no impact of tissue source (p=0.06) or fresh vs thawed processing (p=0.28).

4) FLT3-ITD and IL1-RAP expression

Univariable analysis

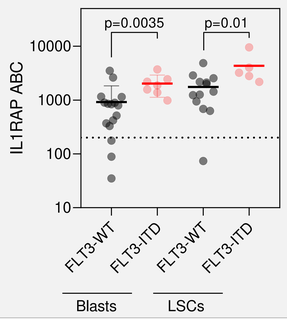
Neither age, sex, ontogeny nor disease status (relapse vs primary refractory), history of intensive Cx, hypomethylating agents, venetoclax or allo-HCT had impact on IL-1RAP ABC on blasts (all p>0.05). Median IL-1RAP ABC of blasts was 1,724 in *NPM1*mut pts vs 856 in *NPM1*wt pts (p=0.04) and 1,878 vs 814 in patients with vs without *FLT3*-ITD pts (p=0.0035, **Figure 3**) while *IDH1/2* mutations had no impact (p=0.91).

Multivariable analysis

In a multivariable linear regression followed by backward variable selection and accounting for sample source, *NPM1* and *FLT3* status, only *FLT3*-ITD status influenced IL-1RAP ABC (p=0.04). All (100%) *FLT3*-ITD pts had an ABC ≥ 200 vs 81% of FLT3wt pts (p=0.5). This was in agreement with FLT3-ITD being the main genetic biomarker associated with increased IL-1RAP expression (FC=1.68, q-val <10-20) in a public transcriptomic dataset of 1224 AML patients (Severens, *Leukemia* 2024).

In LSCs, IL-1RAP ABCs were significantly higher in patients with vs without *FLT3*-ITD (p=0.01, **Figure 3**). All other demographic, treatment history or genetic variables had no impact on IL-1RAP ABC in LSCs.

Figure 3. IL1-RAP according to *FLT3*-ITD status



CONCLUSIONS

- **IL-1RAP is expressed on blasts in 89% of relapsed/refractory AML patients regardless of disease ontogeny and treatment history.**
- **IL-1RAP is over-expressed in phenotypically-defined LSCs compared to blasts.**
- **FLT3-ITD patients have higher IL-1RAP expression in both blasts and LSCs.**

➡ **This data supports the use of IL-1RAP as a target antigen in R/R AMLs. The safety and clinical activity of the first in class, 3rd generation autologous IL- 1RAP-directed CAR T cell therapy (CCTx-001) will be tested in the RESOLVE AML-001 study (NCT06281847).**

CONTACT INFORMATION

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