**CXCL12 UNMET MEDICAL NEED**

- **CXCL12** (Brom-Derived Factor 1, SOFI), play key roles in hemopoiesis, angiogenesis, metastasis, and immune response.
- **CXCL12** signals through 2 main receptors, CXCR4 and CXCR7, activating multiple intracellular G protein-coupled signaling mediators which include PI3K, ROS, NF-kB, JAK/STAT, and MEK/ERK.
- Elevated in some settings, **CXCL12** may also activate CXCR3 and integrins αvβ3, αvβ6, and αvβ1.
- **CXCL12** and its receptors are implicated in cancer progression:
  - Expressed in 85% of breast carcinomas and 45% of prostate cancer tumors to shorter OS (Samardzija 2017).
  - Poor prognosis observed in 40% of PTCL (prolymphocytic leukemia) (Cox 2019).
  - CXCR4 expression is a predictor of poor prognosis in patients with DLBCL (Lambil 2008).
  - 85% of ALCL express high CXCR4 and have poor prognosis (Spicer 2007).
  - CXCR4 is associated with poor prognosis in 82% of ovarian cancer patients (Cox 2016).
- High CXCL12 expression in primary (nipple) breast cancers OS advantage (Samardzija 2017) whereas high CXCR4 overexpression translates to metastasis and reduced survival of 15% of breast cancer patients (Zhong 2011).

**LYMPHOMA INDICATIONS - PTCL**

- The activity of **T cells** in peripheral T cell lymphoma (PTCL) is being investigated in a single agent rituximab (NCT02949628).
- The treatment of **T cell lymphoma** using an antibody that binds to CXCR4 (CXCL12) was found to be effective (NCT02949628).
- Two additional study cohorts are ongoing for **CXCL12-PTCL**: clinical trial NCT03532391 (ongoing).
- **CXCL12** was also recently reported to be overexpressed in 70% of patients with advanced stage PTCL. In this study, CXCR4 expression was observed in 80% of patients with advanced stage PTCL. (Cox 2016).

**LYMPHOMA INDICATIONS – DBLCL, MF**

- The activity of **B cells** in diffuse large B-cell lymphoma (DLBCL) has been studied using a combination of Rituximab (R) and CAR T cells (CAR-T) (Cox 2019).
- **CXCL12** expression was found to be significantly associated with outcomes in DLBCL (Cox 2019).
- **CXCL12** expression was found to be associated with outcomes in DLBCL (Cox 2019).

**HNSCC AND OTHER SQUAMOUS CANCERS**

- **HNSCC** expresses high levels of CXCL12 that are inversely correlated with HRAS-gene expression levels. Higher CXCL12 expression is associated with a poor prognosis. HRAS expression is a predictor of outcome. (Cox 2015). (Bars represent median values), suggesting that HRAS may be downstream of CXCL12/CXCR4 signaling.
- Both wild type and mutant HRAS require farnesyltransferase for membrane localization and function.
- **CXCL12** activity in HNSCC with HRAS-mutations is strongly inhibited in single agent, single arm studies. (Cox 2016).
- **HNSCC** with high CXCR4 expression is highly sensitive to pan-cancer inhibitors like BIBM3344 and GNP-54 (Cox 2017).

**TIPIFARNIB DOWNREGULATES CXCL12**

- **TIPIFARNIB** (dinutuximab) in CD11a mouse bone marrow stromal cultures (Fig. 1A).
- Decrease in **CXCL12** plasma levels in 2 T-cell-related T-cell lymphoma patients (Fig. 1B). TIPIFARNIB dose 300 mg/kit (3 doses of 28-day cycles).
- Gene expression of the uniquely farnesylated RHOE (HRDE) and PRKCD/2K proteins is strongly associated with lung and ovarian tumorigenesis.
- CXCL12 expression is lower in lung tissues compared to ovarian tissues.

**CXCL12 RELATED BIOMARKERS**

- **CXCL12** expression was re-evaluated in 2 patients with triple-negative breast cancer (TNBC) (NCT07387452, patients 1, 2).
- **CXCL12** expression predicted survival (NCT02199325).
- High bone marrow CXCL12 expression was found in AML (Cox 2015).

**MEYLOID INDICATIONS - AML**

- **CXCL12** expression was re-evaluated in 2 patients with triple-negative breast cancer (TNBC) (NCT07387452, patients 1, 2).
- High bone marrow CXCL12 expression was found in AML (Cox 2015).

**CONCLUSIONS**

- Disruption of the **CXCL12** pathway is observed in a large number of oncology indications and consolidates an unmet medical need. Approximately 30-50% of patients with myeloid neoplasms, lymphomas, and solid tumor indications could be reached with **CXCL12** pathway disruption.
- **TIPIFARNIB** is a potent and selective farnesyltransferase inhibitor that downregulates **CXCL12** in tumor models and cancer patients. Potential farnesyltransferase targets mediating this effect are currently under investigation.
- Proof-of-concept for **TIPIFARNIB** as a **CXCL12** inhibitor was reached in a T cell lymphoma study.
- **CXCL12** pathway biomarkers may identify pre-susceptible to receive clinical benefit from **TIPIFARNIB** therapy and could enable registration strategies for this agent in HNSCC, PTCL, CTCL, EBLSC, AML, and pancreatic cancer, among other indications.

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Mechanism of Action of the Farnesyltransferase Inhibitor Tipifarnib and its Potential Clinical Applications

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CXCL12 UNMET MEDICAL NEED

- CXCL12 (Stroma Derived Factor 1, SDF1), play key roles in hematopoiesis, angiogenesis, neurogenesis, and immune response.
- CXCL12 signals through 2 main receptors, CXCR4 and CXCR7, activating heterotrimeric G proteins. Downstream signaling molecules include PI3K, RAS isoforms, JAK/STAT and GRK/β-Arrestin. In some settings, CXCL12 may also activate CXCR3 and integrins αvβ3, α4β1, and α5β1.
- CXCL12 and its receptors are implicated in cancer progression:
  - Expressed in 50% esophagogastric and 49% pancreatic cancer translating to shorter OS (Samarendra 2017).
  - Poor prognosis observed in 40% of PTCL overexpressing CXCL12 (Witzig 2019).
  - CXCL12 expression is part of a stromal poor prognosis signature in DLBCL (Lenz 2008).
  - 35% AML express high CXCR4 and carry poor prognosis (Spoo 2007).
  - CXCR4 is associated with poor prognosis in 61% of ovarian cancer pts (Liu 2014).
  - High CXCL12 expression in primary (surgically treated) tumors confers OS advantage (Samarendra 2017) whereas high CXCR4 overexpression translates to metastasis and reduced survival in 36% of breast cancer pts (Zhang 2014).
Tipifarnib downregulates CXCL12 secretion ex-vivo in CD1 mouse bone marrow stroma cultures (FIG. 1A).

- Decrease in CXCL12 plasma levels in 2 tipifarnib treated T-cell lymphoma pts (FIG. 1B, tipifarnib dose 300 mg bid for 21 of 28-day cycles).
- Gene expression of the uniquely farnesylated RHOE (RND3) and PRICKLE2 proteins is strongly associated with bone marrow stroma (VCAM+) CXCL12 expression, suggesting potential CXCL12-related tipifarnib targets (FIG. 1C). Data from TCGA, AML, Provisional.

CXCL12 (PTCL, other lymphomas)
- MMPs, DPP IV?
- 5-67 CXCL12
  - CXCR4
    - KRAS* (Pancreatic cancer, other CXCL12-dependent solid tumors)
  - CXCR3
    - HRAS* (HNSCC, other SCC)
    - (AML, other myeloid indications)
LYMPHOMA INDICATIONS - PTCL

- The activity of Tipifarnib in Peripheral T cell lymphoma (PTCL) is being investigated in a single agent Ph 2 trial. NCT02464228.
- Pre-treatment tumor CXCL12 expression was identified as a biomarker of clinical benefit from tipifarnib in a preliminary retrospective analysis (N=24). (FIG. 3).
- Two additional study cohorts enriching for CXCL12-expressing PTCL continue to investigate this hypothesis prospectively. The first of these cohorts in angioimmunoblastic lymphoma pts has reached proof-of-concept with 40% ORR (Witzig 2018).
- CXCL12 was also retrospectively investigated in 73 pts who received standard of care therapy. A trend for poor prognosis (HR=1.8, p=0.09) was observed at CXCL12 expression level associated with better outcome in tipifarnib treated pts.
LYMPHOMA INDICATIONS – DLBCL, MF

- The activity of tipifarnib in relapsed/refractory lymphomas was previously investigated in a single agent Ph 2 trial (N=93; NCT00082888, Witzig 2011).

- Pre-treatment tumor samples and best response data were obtained from 20 pts: 6 Diffuse Large B Cell Lymphoma (DLBCL), 6 Hodgkin Lymphoma (HL), 4 Follicular Lymphoma (FL), 1 Marginal Zone B Cell Lymphoma, 1 PTCL NOS, 2 Mycosis Fungoides (MF).

- 3 PRs were reported in DLBCL, 1 in HL and 2 in MF in this subset.

- Pre-treatment tumor CXCL12 expression was identified as a potential marker of clinical benefit in DLBCL. High pre-treatment CXCL12 (FIG. 4A), CXCL12/CXCR4 Expression Ratio (FIG. 4B), and CXCL12/CXCR7 Expression Ratio (not shown) predicted objective response.

- Two out of 2 MF pts with high pre-treatment tumor CXCL12 expression (CXCL12/CXCR4= 0.6, 2.0) experienced PRs.

- No relationship between CXCL12 expression and clinical benefit in HL was observed in this dataset.

FIG. 4A and B. Objective responses in tipifarnib treated DLBCL pts with high tumor CXCL12 expression as detected by RNA Seq.
· CXCL12 biomarkers were retrospectively investigated in 2 tipifarnib Ph2 AML studies: CTEP20 (elderly, unfit AML; NCT00027872; Lancet 2007) and INT-17 (relapsed/refractory AML; NCT00354146; Harousseau 2007).

· High bone marrow CXCL12 expression was found in AML pts experiencing clinical benefit from tipifarnib treatment (Gualberto 2017).

· CXCL12 may also bind CXCR3 upon chemokine cleavage by proteases generating a 5-67 CXCL12 fragment (Vergote 2006). High CXCR3 expression was also a significant predictor of PFS and OS benefit from tipifarnib in CTEP20 and INT-17 (not shown).

· The product of CXCL12 and CXCR3 expression was highly predictive of CR in study CTEP20 (FIG. 5)

· Other AML settings of interest include front line treatment in combination with idarubicin and cytarabine (Jabbour 2011) and maintenance therapy (Luger 2015).
HNSCC AND OTHER SQUAMOUS CANCERS

- HNSCC expresses low levels of CXCL12 that are inversely correlated with HRAS gene expression and HRAS mutant variant gene allele frequency (FIG. 6A. Data from TCGA, HNSCC, Provisional. Bars represent median values), suggesting that HRAS may be downstream from CXCL12/CXCR4 signaling.
- Both wild type and mutant HRAS require farnesylation for membrane localization and function.
- Tipifarnib activity in HNSCC with HRAS mutations is being investigated in single agent, single arm trials (NCT02383927, NCT03719690).
- HNSCC with >35% HRAS mutant variant allele frequency (VAF) or ≥20% VAF if serum albumin is ≥3.5 g/L was found to be particularly sensitive to tipifarnib therapy (FIG. 6B) (Ho 2018).
A Ph3 trial of gemcitabine + tipifarnib vs gemcitabine + placebo was conducted in previously untreated advanced pancreatic cancer pts. Median OS were 6.4 vs 6.1 months (NCT00005648; Van Cutsem, 2004).

FIG. 7A: Subset analysis based on the reported association between high CXCL12 expression and attenuation of abdominal pain (Demir 2017). Absence of pain translated to 4 months survival benefit in the tipifarnib arm.

FIG. 7B: Analysis based on expected high CXCL12 expression in liver metastases (Liepelt 2016). Pts with liver metastasis experienced a 1.8 month improvement in OS with tipifarnib.

FIG. 7C: Pancreatic cancer expresses high levels of CXCL12 that are inversely correlated with KRAS gene expression and KRAS mutant VAF, suggesting that low KRAS mutant VAF (<5% VAF, 31% pancreatic pts) may serve as a surrogate of high CXCL12 expression and sensitivity to tipifarnib. KRAS differs from HRAS in its alternative forms of prenylation. Data from TCGA, Pancreatic cancer, provisional.
CONCLUSIONS

- Dysregulation of the CXCL12 pathway is observed in a large number of oncology indications and constitutes an unmet medical need. Approximately 30-50% of pts with myeloid neoplasia, lymphoma, and solid tumor indications course with CXCL12 pathway dysregulation.

- Tipifarnib is a potent and selective farnesyltransferase inhibitor that downregulates CXCL12 in tumor models and cancer patients. Potential farnesylated targets mediating this effect are currently under investigation.

- Proof-of-concept of tipifarnib as a CXCL12 inhibitor was reached in a T cell lymphoma study.

- CXCL12 pathway biomarkers may identify pts susceptible to receive clinical benefit from tipifarnib therapy and could enable registrational strategies for this agent in HNSCC, PTCL, CTCL, DLBCL, AML, and pancreatic cancer, among other indications.
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