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

Antonio Gualberto[1], Catherine Scholz[1], Vishnu Mishra[2], Matthew R. Janes[3], Linda Kessler[2], Eric Van Cutsem[4], Alan Ho[5], Thomas Witzig[6]

[1]Kura Oncology, Inc., Cambridge, MA; [2]Kura Oncology, Inc., San Diego, CA; [3]Wellspring Biosciences, Inc., San Diego, CA; [4]University Hospitals Leuven, Belgium; [5]Memorial Sloan Kettering Cancer Center, New York, NY; [6]Mayo Clinic Foundation, Rochester, MN

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).
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- 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).
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FIG. 3

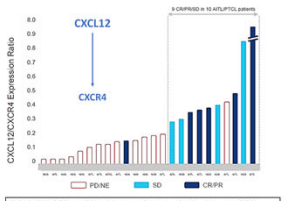

FIG. 3. 50% ORR and 90% clinical benefit rate in 10 tipifarnib treated PTCL subjects with high tumor CXCL12 expression as detected by RNA Seq.

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).

FIG. 6A

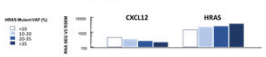

FIG. 6B

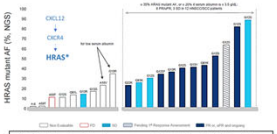

FIG. 6B. High rate of objective responses in tipifarnib treated SCC patients with high HRAS mutant VAF as determined by next generation sequencing.

TIPIFARNIB DOWNREGULATES CXCL12

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- 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.

FIG. 1A

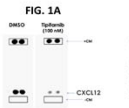


FIG. 1B

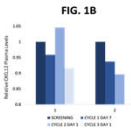
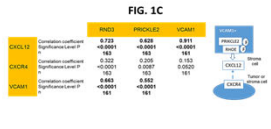


FIG. 1C



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

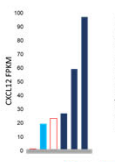


FIG. 4B

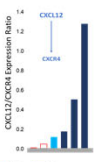


FIG. 4A and B. Objective responses in tipifarnib treated DLBCL pts with high tumor CXCL12 expression as detected by RNA Seq.

PANCREATIC CANCER

- 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.

FIG. 7A

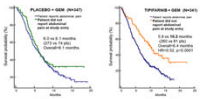


FIG. 7B

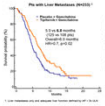
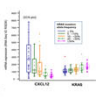


FIG. 7C



CXCL12 RELATED BIOMARKERS

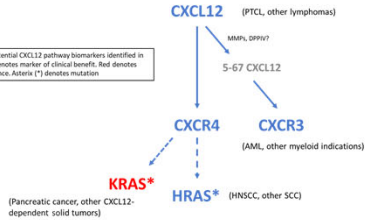


FIG. 2. Summary of potential CXCL12 pathway biomarkers identified in tipifarnib trials. Blue denotes marker of clinical benefit. Red denotes marker of drug resistance. Asterix (*) denotes mutation

MYELOID INDICATIONS - AML

- 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).
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FIG. 5

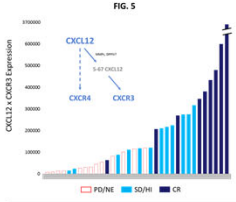

FIG. 5. High rate of CR in tipifarnib treated elderly, unfit AML pts with high levels of CXCL12 and CXCR3 expression as determined by gene chip assays

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.

References

Chatterjee 2014. Adv Cancer Res. 124: 31-82
Demir 2017. PNAS USA 114:E85-E94
Gualberto 2017. Blood 130:3957
Harousseau 2007. Blood 109:5151-5156
Ho 2018. Annals Onc 29: viii372-99
Jabbour 2011. Cancer 117:1236-44
Lancet 2007. Blood 109:1387-94
Lee 2011. Exp Ther Med. 2:499-504
Lenz 2008. N Engl J Med 2008; 359:2313-23
Liepelt 2016. Am J Physiol Gastrointest Liver Physiol 311: G203–G209
Liu 2014. PLoS ONE 9: e92629.
Luger 2015. Blood 2015 126:1308
Spoo 2007. Blood 109:786-91
Van Cutsem 2004. J Clin Oncol. 22:1430-8.
Vergote 2006. PNAS USA 103:19182-7
Witzig 2011. Blood 118:4882-9
Witzig 2018. ASH #27517
Zhang 2014. BMC Cancer 14:49-57

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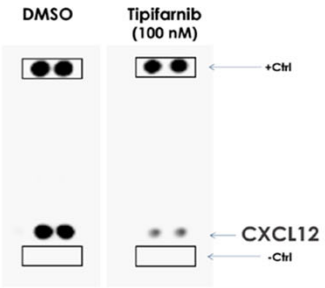


FIG. 1B

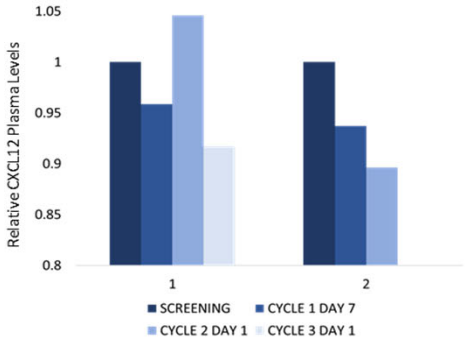
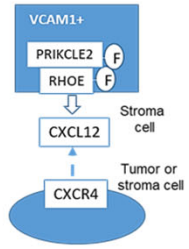


FIG. 1C

		RND3	PRICKLE2	VCAM1
CXCL12	Correlation coefficient	**0.723**	**0.628**	**0.911**
	Significance Level P	**<0.0001**	**<0.0001**	**<0.0001**
	n	**163**	**163**	**161**
CXCR4	Correlation coefficient	0.322	0.205	0.153
	Significance Level P	<0.0001	0.0087	0.0520
	n	163	163	161
VCAM1	Correlation coefficient	**0.663**	**0.552**	
	Significance Level P	**<0.0001**	**<0.0001**	
	n	**161**	**161**	



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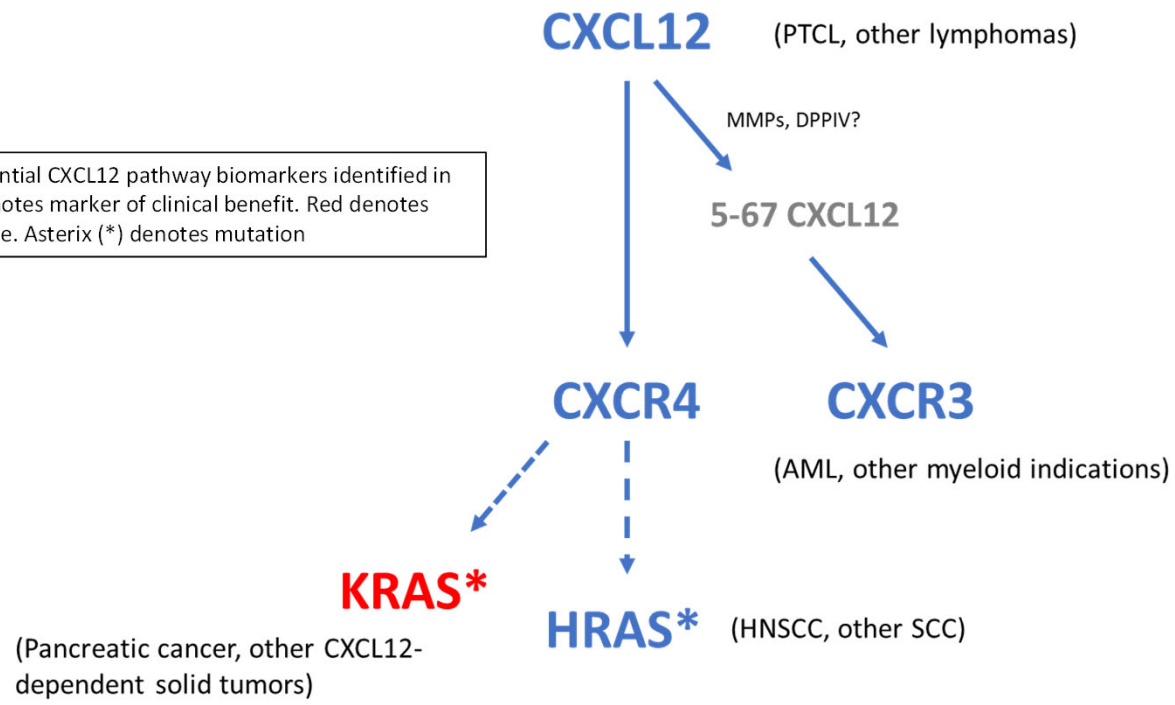

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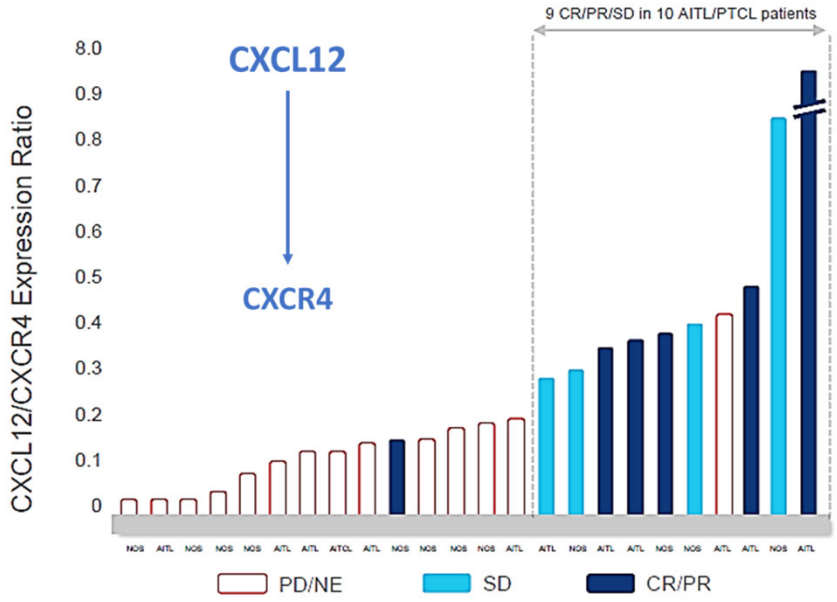

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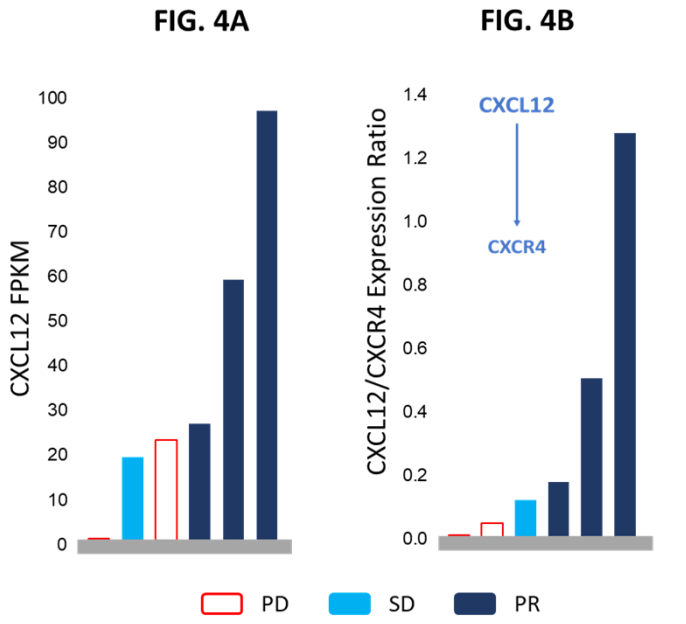

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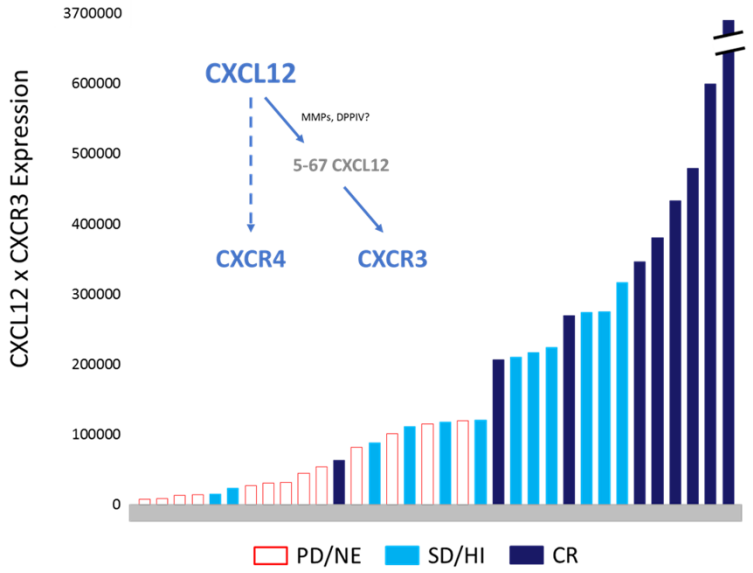

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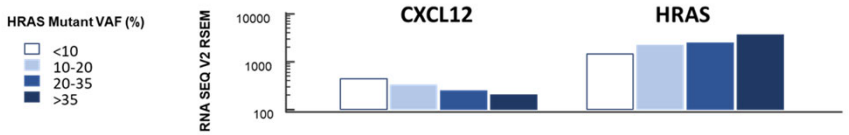

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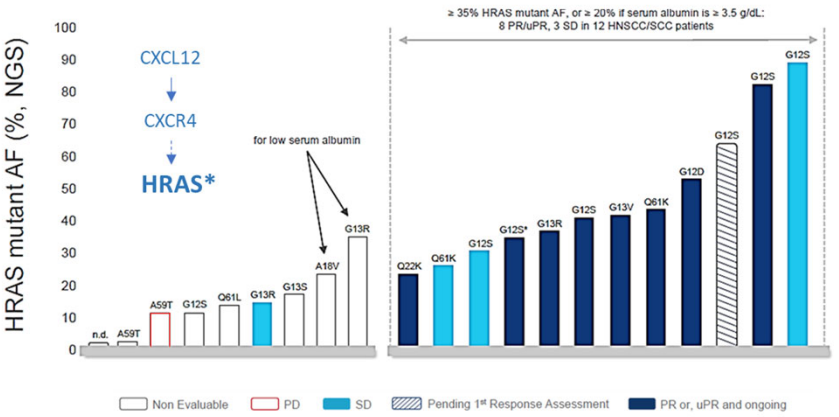

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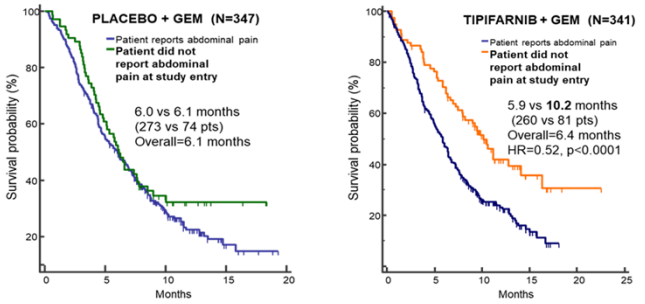

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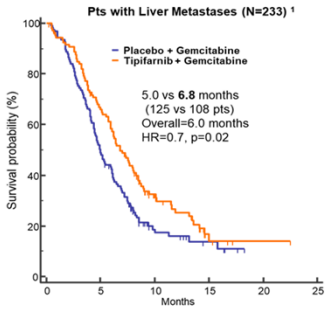
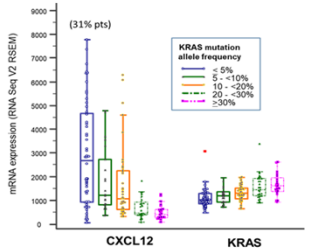


1. Liver metastases only and adequate liver function defined by AP < 3x ULN

FIG. 7C



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.

References

Chatterjee 2014. Adv Cancer Res. 124: 31-82
Demir 2017. PNAS USA 114:E85-E94
Gualberto 2017. Blood 130:3957
Harousseau 2007. Blood 109:5151-5156
Ho 2018. Annals Onc 29: viii372-99
Jabbour 2011. Cancer 117:1236-44
Lancet 2007. Blood 109:1387-94
Lee 2011. Exp Ther Med. 2:499-504
Lenz 2008. N Engl J Med 2008; 359:2313-23
Liepelt 2016. Am J Physiol Gastrointest Liver Physiol 311: G203–G209
Liu 2014. PLoS ONE 9: e92629.
Luger 2015. Blood 2015 126:1308
Spoo 2007. Blood 109:786-91
Van Cutsem 2004. J Clin Oncol. 22:1430-8.
Vergote 2006. PNAS USA 103:19182-7
Witzig 2011. Blood 118:4882-9
Witzig 2018. ASH #2937
Zhang 2014. BMC Cancer 14:49-57