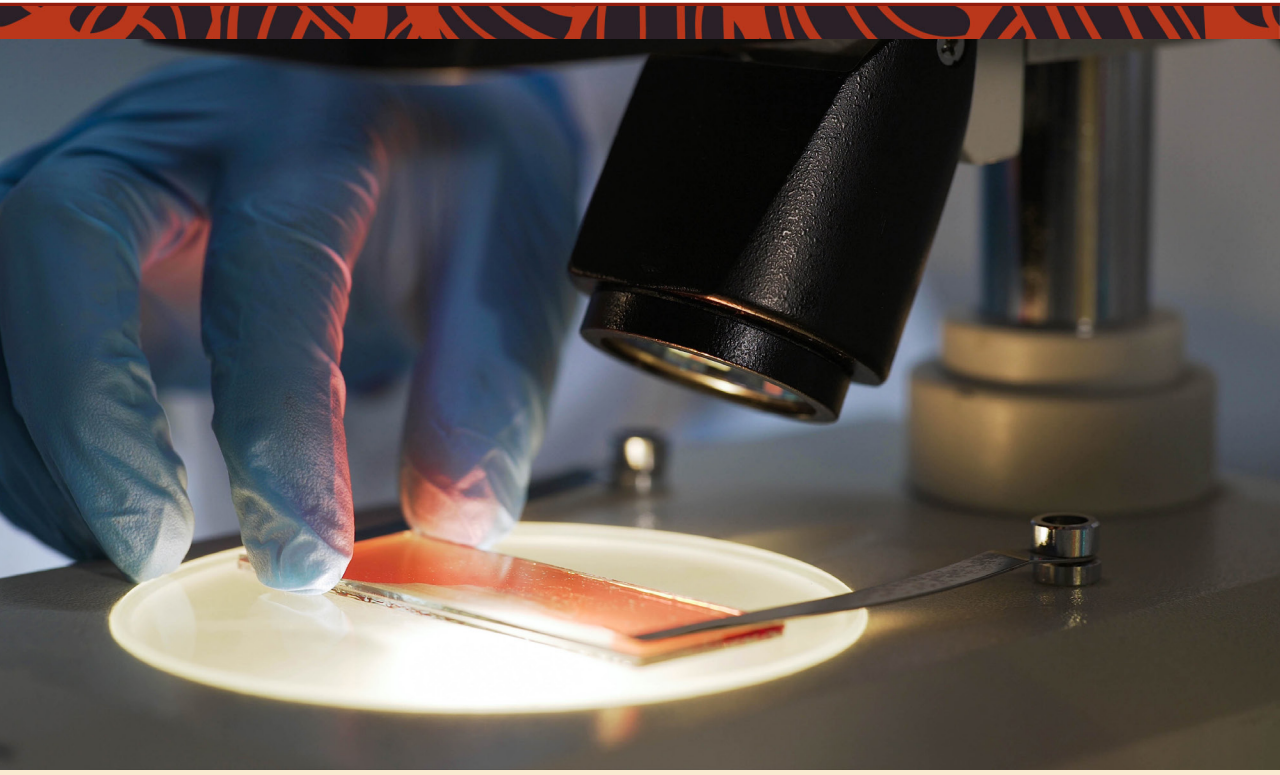
MPN Interferon Initiative

Overall Scientific Summary



MPN RESEARCH FOUNDATION

The Interferon Initiative was a three-year, multi-institutional investigation by prominent researchers across the US, Europe and Australia to uncover a deeper understanding of the mechanisms by which interferon works, and sometimes doesn't work, for patients with a myeloproliferative neoplasm (MPN).

www.mpnresearchfoundation.org/mpn-interferon-initiative/

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INTRODUCTION

In real world usage and in clinical trials, interferon (IFN), in a variety of forms, has shown high rates of hematological and molecular responses in most patients with polycythemia vera (PV) and essential thrombocythemia (ET), and some patients in the early phase of primary myelofibrosis (PMF). This is great news but there is still much to learn. Some patients do not have a significant response, some develop drug resistance over time, and others experience a relatively long treatment period culminated by a complete molecular response. The reasons for these varying responses remain obscure. This is mostly due to the fact that the molecular mechanisms by which recombinant IFNa (rIFNa) reduces the mutant JAK2 allele burden are elusive.

It falls within the MPN Research Foundation's mission to uncover a deeper understanding of the mechanism(s) by which a drug works for patients with a myeloproliferative neoplasm (MPN). To that end, the MPN IFN Initiative was launched in December 2017 as a multi-institution, multi-year collaborative effort to bring together global IFN experts to study the underlying mechanism of action of IFN and better define the patient/disease profile that predicts both response to treatment and/or resistance to therapy. This understanding can help to validate the use of newer, more disease-specific forms of IFN as well as new approaches to more rational IFN-based drug combinations for MPN patients. The potential to define new downstream IFN signaling targets that can be modulated to enhance the activity of IFN also remains promising.

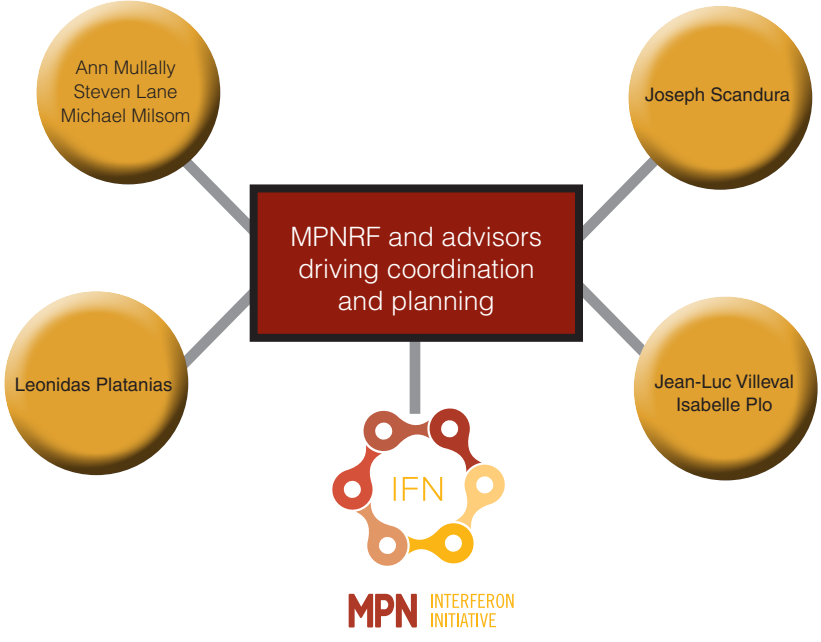
Specifically, the goals of the IFN Initiative were to:

- Further understand the mechanism of action of IFN especially as it relates to MPN stem and progenitor cell function;
- Mechanistically understand why some patients respond and others do not;
- Discover what factors lead to IFN resistance over time and how to overcome that resistance; and
- Develop strategies to enhance the activity of IFN or identify new therapeutic options based on IFN signaling pathways.

Resolving these questions has the potential to have a major impact, not only for MPNs, but also for other blood cancers and solid tumors.

IFN INITIATIVE STRUCTURE

The IFN Initiative had four active projects led by the principal investigators (PIs) shown in the figure below.



The PIs worked closely with MPNRF staff and presented project updates annually to a distinguished international group of academic advisors to track progress, solicit feedback and promote collaboration. The group of advisors included:

- Andrew Schafer, MD (Weill Cornell)
- John Crispino, PhD (St. Jude)
- Robert Cohen, MD (Calico Life Sciences)
- Ron Hoffman, MD (Icahn School of Medicine at Mt. Sinai)
- Richard Silver, MD (Weill Cornell)
- Jean-Jacques Kiladjian, MD (Saint-Louis Hospital and Paris Diderot University)
- Radek Skoda, PhD (University of Basel)
- William Vainchenker, MD, PhD (INSERM, Gustave Roussy)
- Hans Hasselbalch, MD, PhD (Zealand University Hospital)
- Josef Prchal, MD (University of Utah)

PROJECT SUMMARIES

The research activities for the Initiative were completed in March 2021. A brief summary of the projects and research findings are presented below. For more details, see the abstracts or publications listed at the end of each summary.

Overcoming Resistance to Interferon in MPN Stem Cells – Ann Mullally, MD (Brigham and Women's Hospital, Boston MA); Steven Lane, MD, PhD (QIMR Berghofer Medical Research Institute, Brisbane, Australia); and Michael Milsom, PhD (German Cancer Research Center, Heidelberg, Germany)

Three fundamental questions were addressed: 1) Do epigenetic or other relevant secondary mutations in MPN stem cells mediate resistance to IFN? 2) Why are MPN stem cells preferentially sensitive to IFN? and 3) What are the key genetic drivers of resistance to IFN in MPN patients? The team developed new mouse models of MPNs and used patient samples from a large clinical trial to address these questions.

A JAK2/DNMT3A double mutant mouse model was developed to compare the effect of murine pegylated rIFN alpha (pegrIFNa) in the double mutant model versus the standard JAK2 mutant only model. Mutations in DNMT3A did not modify rIFN treatment outcomes compared to the standard JAK2 mutant model and IFN was similarly effective in inducing a loss of hematopoietic stem/progenitor cell quiescence in both models. With the anticipated rIFN resistance not observed in the JAK2/DNMT3A model, additional secondary mutation targets were explored by performing bulk RNA sequencing on highly purified long-term hematopoietic stem cell (HSC) populations. Preferentially enriched gene signatures relating to cell cycle progression, p53 stabilization and EZH2 targets were found before and after rIFN treatment suggesting that concomitant mutations in either TP53 or EZH2 may contribute to rIFN resistance through maintenance of quiescent stem cells. Therefore, a JAK2/TP53 double mutant mouse model was developed, leading to PV in four weeks and ultimately progressing to leukemia. rIFN treatment studies are now underway and will continue beyond the scope of this funding. Additionally, a JAK2/EZH2 double mutant model is under development to further explore secondary epigenetic mutation candidates.

To further study stem cell sensitivity to IFN, HSC label retention time course experiments were performed in a JAK2 mutant mouse model of PV to study the phenomenon of stem cell dormancy.

These studies demonstrated the existence of dormant JAK2-mutant HSCs that are resistant to JAK2 inhibitor treatment. However, pegrIFNa was able to deplete these dormant mutant HSCs, perhaps explaining its ability to induce molecular remission in some patients. Mechanism of action studies indicate that rIFN induces DNA damage in these HSCs via induction of reactive oxygen species which may lead to their eventual elimination. Preliminary experiments are underway testing novel drug combinations with pegrIFNa to further enhance its activity in mutant stem cell depletion and this work will be continued via the DKFZ (German Cancer Research Center) fellowship that was secured by Dr. Milsom.

To study potential genetic drivers of rIFN resistance in patients, targeted next-generation sequencing (NGS) was performed on 202 pre-treatment samples obtained from patients with an MPN enrolled in the DALIAH trial (randomized controlled phase III clinical trial) and 135 samples obtained after 24 months of therapy with rIFNα or hydroxyurea (HU). The primary aim was to evaluate the association between complete clinicohematologic response (CHR) at 24 months and molecular response obtained through sequential assessment of 120 genes using NGS. It was found that among patients with a JAK2 mutation treated with rIFNα, those with CHR had a greater reduction in the mutant JAK2α variant allele frequency (VAF) compared with those not achieving CHR. In contrast, the mutant calreticulin (CALR) VAF did not significantly decline in either those patients achieving CHR or in those not achieving CHR. Treatment-emergent mutations in DNMT3A were observed more commonly in patients treated with rIFNα compared with HU and treatment-emergent DNMT3A-mutations were significantly enriched in rIFNα treated patients not attaining CHR. More details on the distinct patterns of response to rIFNα in JAK2-mutant MPN as compared with CALR-mutant MPN can be found in abstract form from the ASH Annual Meeting in 2019. https://ashpublications.org/blood/article/134/Supplement_1/4202/425637/Genomic-Profiling-of-a-Phase-III-Clinical-Trial-of?searchresult=1. Overall these results provide some insights into molecular response and resistance to rIFN and provide additional insight for the clinical use of rIFN in MPN patients.

PUBLICATION SUMMARY

Jacquelin et al, 2018, Jak2V617F and Dnmt3a Loss Cooperate to Induce Myelofibrosis Through Activated Enhancer-Driven inflammation. **Blood:** https://pubmed.ncbi.nlm.nih.gov/30366920/, DOI: 10.1182/blood-2018-04-846220

Austin et al, 2020, Distinct Effects of Ruxolitinib and Interferon-Alpha on Murine JAK2V617F Myeloproliferative Neoplasm Hematopoietic Stem Cell Populations. **Leukemia:** https://pubmed.ncbi.nlm.nih.gov/31732720/, DOI: 10.1038/s41375-019-0638-y

Knudsen et al, 2021, Genomic Profiling of a Randomized Trial of Interferon-A Versus Hydroxyurea in MPN Reveals Mutation-Specific Responses. **Blood Advances:** https://doi.org/10.1182/bloodadvances.2021004856

Mechanism of Action of Interferon Alpha in MPN Therapy – Jean-Luc Villeval, PhD and Isabelle Plo, PhD (INSERM/Gustave Roussy/University of Paris-Saclay, France)

This project was focused on the mechanism of action of IFN by 1) testing hypotheses on the interplay between mutant JAK2 (i.e., JAK2V617F) and IFN signaling, and for a potential role of PML-nuclear bodies in enhancing this interplay; 2) evaluating the activity of IFN in JAK2 versus CALR mutated cells; and 3) developing an algorithm to predict the long term IFN response in patients.

It was learned that the JAK2V617F mutation sensitizes IFN pathway signaling much better than CALR mutations. Interestingly, the combination of a JAK2 specific inhibitor with IFN mildly increases IFN target gene expression in vitro, suggesting that the hematological and molecular response induced by IFN in mice may benefit from a combination with a type I JAK2 inhibitor. The results with IFN and JAK2 inhibitors in preclinical models are somewhat paradoxical but suggest a potential benefit of this combination for some patients. It was also shown that IFN uses specific nuclear structures called PML-nuclear bodies to eliminate the JAK2V617F malignant clone and that this action can be amplified if IFN is combined with arsenic trioxide, a drug that increases PML-nuclear body tumor repressor functions. These results have led to the planning of IFN and arsenic based clinical trial approaches for MPN patients. In addition, a CALR mutation mouse model developed by this team is being used to investigate if this combination may also benefit patients with a CALR mutation.

Lastly, blood samples from a prospective clinical study over a five-year period, including 48 patients treated with rIFN, were analyzed to determine the long-term molecular efficacy of rIFN on human disease-initiating HSCs. Their results indicate that rIFN can slowly eradicate mutated HSCs but more efficiently in patients with a homozygous compared to a heterozygous JAK2V617F mutation, especially if treated with a high dose of IFN. The response of CALR mutated HSCs to IFN was more heterogeneous depending on type 1 or type 2 CALR mutations, and a higher dose of IFN actually correlates with worse outcome in this study. Together, this work suggests that the long-term molecular efficacy of rIFNα depends on both the driver mutation type and rIFNα dosage and implies an HSC exhaustion mechanism of action. These results may be useful to better stratify patients for IFN treatment.

PUBLICATION SUMMARY

Numerous abstracts, oral presentations and publications have resulted from the work of this group. A summary of the full manuscript publications is below.

Dagher et al, 2021, JAK2V617F Myeloproliferative Neoplasm Eradication By a Novel Interferon/Arsenic Therapy Involves PML. **J. Exp. Med:** https://pubmed.ncbi.nlm.nih.gov/33075130/

Toppaldoddi et al., 2018, Rare Type 1-Like and Type 2-Like Calreticulin Mutants Induce Similar Myeloproliferative Neoplasms as Prevalent Type 1 and 2 Mutants In Mice. **Oncogene:** https://pubmed.ncbi.nlm.nih.gov/30846848/

Benlabiod et al, 2020, Calreticulin Del52 and Ins5 Knock-In Mice Recapitulate Different Myeloproliferative Phenotypes Observed in Patients with MPN. **Nature Communications:** https://www.nature.com/articles/s41467-020-18691-3

Campario et al, 2020, Impact of Interferon On a Triple Positive Polycythemia Vera. **Leukemia:** https://pubmed.ncbi.nlm.nih.gov/31728058/

Mosca et al, 2021, Inferring the Dynamic of Mutated Hematopoietic Stem and Progenitor Cells Induced by IFNα in Myeloproliferative Neoplasms. **Blood:** https://doi.org/10.1182/blood.2021010986

Benlabiod, et al, 2021, Lessons From Mouse Models of MPN. **International Review of Cell and Molecular Biology:** https://doi.org/10.1016/bs.ircmb.2021.02.009

Novel Agents For the Treatment of Malignancies – Leonidas Platanias, MD, PhD (Northwestern University Feinberg School of Medicine, Chicago IL)

The goal of this project was to identify and characterize novel proteins that participate in the generation of the IFN response in cells of MPN patients and to determine the biological effects of targeting these candidate IFN response proteins alone and in combination with IFN treatment. Previous studies have shown that ULK1 was a key protein in the IFN signaling pathway needed for its anti-neoplastic activity and is upregulated in MPN patients. In this project, two additional candidate interacting proteins were identified using mass spectrometry screening analysis in JAK2V617F expressing human erythroleukemia (HEL) cells: Lim domain-binding protein 1 (LDB1) and Chromatin assembly factor 1B (CHAF1B). Importantly, the gene expression of these proteins is also upregulated in the blood of MPN patients, compared to healthy controls.

LDB1 is a hematopoietic adaptor protein which controls HSC maintenance, including the differentiation of erythroid cells and megakaryocytes from the megakaryocyte-erythroid progenitors. The interaction between ULK1 and LDB1 was validated in the JAK2V617F expressing cell lines, SET-2 and HEL cells. Gene expression of LDB1 was especially upregulated in ET patients, compared to healthy controls, and was required for cellular proliferation and viability of the ET-derived SET-2 cell line. Additionally, knocking down of LDB1 levels enhanced IFN-induced anti-proliferative effects in these cells, suggesting that ultimately targeting LDB1 might be more relevant in ET patients.

CHAF1B is essential for normal hematopoiesis, but its overexpression promotes leukemia. ULK1 and CHAF1B also interact in SET-2 and HEL cells and knockdown of CHAF1B increased IFN-expression of several IFN stimulated genes (ISGs) in these cells. It also enhanced IFN-induced antineoplastic effects in JAK2V617F expressing cell lines and in primary erythroid progenitor cells from PV patients. Dr. Platanias' working hypothesis is that CHAF1B prevents the binding of ISG specific transcription factors to the promoter region of ISGs, thus blocking the induction of IFN-mediated anti-neoplastic responses. Current plans are to develop compounds targeting this protein which may enhance the ability of IFN to block the growth of MPN cells.

In summary, two unique proteins were identified that are overexpressed in MPN patients, the targeting of which enhances anti-neoplastic effects of IFN against MPN cells. These proteins seem to work as negative feedback regulators of the IFN response, thus eventually halting its anti-tumor effects in MPNs. These studies should provide the basis for future development of compounds targeting these proteins that may prove effective in the treatment of MPNs alone or in combination with IFN.

PUBLICATION SUMMARY

A manuscript covering this work is in progress and should be submitted for publication in the near future. Listed below are several presentations given at local and international conferences.

Saleiro D., 2019, Novel Interferon Signaling Targets in Myeloproliferative Neoplasms. 3rd International Conference on Cytokine Signaling in Cancer. Rhodes, Greece.

Saleiro D., 2019, Novel Interferon Signaling Targets in Myeloproliferative Neoplasms. 11th Annual Lurie Cancer Center Symposium, Robert H. Lurie Comprehensive Cancer Center of Northwestern University. Chicago, IL, USA.

Platanias LC., 2020, Interferon Signaling in Malignancies. First Annual Tina Mantis Lecture, Robert H. Lurie Comprehensive Cancer Center. Chicago, IL, USA.

Using a Vascular Niche Platform to Develop Interferon-Based Strategies to Eradicate MPN Stem Cells and Phenotypes – Joseph Scandura, MD, PhD (Weill Cornell Medicine, New York, NY)

The goal of this project was to track the strength or "fitness" of MPN stem cells (i.e., the ability of MPN stem cells to outcompete their normal counterparts) and to develop ways to weaken these malignant stem cells. Dr. Scandura developed a "cell-based blood formation factory" that allows stem cells to be grown and interrogated in the laboratory. This platform offered an opportunity to quickly and robustly assess the relative expansion of MPN and normal HSCs simultaneously in a well-controlled experimental setting. By measuring the fitness of MPN stem and progenitor cells (HSPCs) in patient samples, the goal was to determine the proportion of IFN activity that is mediated through its action on normal hematopoietic cells, on MPN cells, on the bone marrow microenvironment, or on some combination of these.

By quantifying the mutant allele frequency of 11 well-defined HSPC and mature blood cell populations purified from routinely collected blood and marrow specimens, Dr. Scandura was able to show that MPN cell fitness is patterned and linked to the clinical phenotype. He demonstrated four predominant patterns with which JAK2V617F mutated cells outcompete normal HSPCs across all MPN subtypes, and that these patterns are linked to risk of adverse events such as progression or thrombosis. Using his model, changes in MPN stem and progenitor cell fitness can be measured and can help to predict both negative events such as progression and desired events such as response to drug therapy. It was also shown that MPN stem cells may progress (i.e. outcompete) normal HSCs even in the setting of a clinical and molecular response within whole blood.

Regarding the use of the vascular niche platform to interrogate IFN signaling effects in MPN HSCs, it was shown that the vascular niche platform can be scaled to allow high-throughput studies using primary human samples and to efficiently test novel agents/drug combinations targeting MPN HSPCs ex vivo. For example, IFN can reduce MPN HSPC fitness in some, but not all, patients' samples when tested in this system. Again, whole blood and MPN HSPC molecular responses don't always agree, which may partially explain why the effects of IFN may not be permanent in some patients. Overall, this platform has the potential to speed up and simplify the discovery of agents with the promise of disease modifying activity.

PUBLICATION SUMMARY

Some of this work was presented at the American Society for Hematology meetings in 2018, 2019 and 2020. A full publication and pre-print covering this work can be found below.

Abu-Zeinah et al, 2021, Hematopoietic Fitness of JAK2^{V617F}Myeloproliferative Neoplasms is Linked to Clinical Outcome. **MedRxiv:** https://www.medrxiv.org/content/10.1101/2021.01.28.21250575v1

Abu-Zeinah et. al, 2021, Interferon-Alpha For Treating Polycythemia Vera Yields Improved Myelofibrosis-Free and Overall Survival. **Leukemia:** https://pubmed.ncbi.nlm.nih.gov/33654206/

CONCLUSION

Notable progress was made toward many of the goals of MPNRF's Interferon Initiative. One of the goals was to promote collaboration among IFN researchers. Through frequent calls and annual meetings, this group of researchers developed closer relationships, shared ideas and reagents, and will hopefully pursue future collaborative projects as a result. Through this initiative researchers were able to gain access to murine ropeginterferon, which enabled higher level mouse studies to be completed. Dr. Milsom was relatively new to the IFN research area and we are very pleased that he plans to continue this research.

The Interferon Initiative encouraged the development of several important new double mutant mouse models and the further exploration of an in vitro blood formation system, MPN cell lines and patient samples. All of these developments are integral to make progress in our understanding of IFN signaling and its unique targeting of quiescent JAK2 mutant stem cells, its cross talk with JAK2V617F signaling, the role of PML nuclear bodies, and other potential downstream interacting proteins. This work should lead to more rational drug combination trials, such as IFN and arsenic trioxide, and potentially new drug targets to enhance the activity of IFN. Furthermore, we are now better positioned to understand the role of secondary mutations on IFN resistance and relapse and to develop therapeutic strategies to circumvent these effects. Lastly, we are closer to optimizing IFN treatment strategies based on a patient's specific driver mutation, allele burden and overall genomic profile.

We are pleased the principal investigators indicated that much of this work will continue beyond the scope of this specific funding, and we anticipate that the support by the MPNRF and others who contributed to the effort have positioned these investigators well for future funding opportunities.

PROJECT SUPPORT

It is important to note that funding support for this project came from multiple sources in addition to funding from the MPN Research Foundation. Dr. Steven Lane was supported in part by MPN Australian Alliance. Dr. Joseph Scandura's project was funded by CR&T, the Cancer Research & Treatment Fund. We also gratefully acknowledge the financial support of PharmaEssentia.