How to unmask unique vulnerabilities in leukaemia

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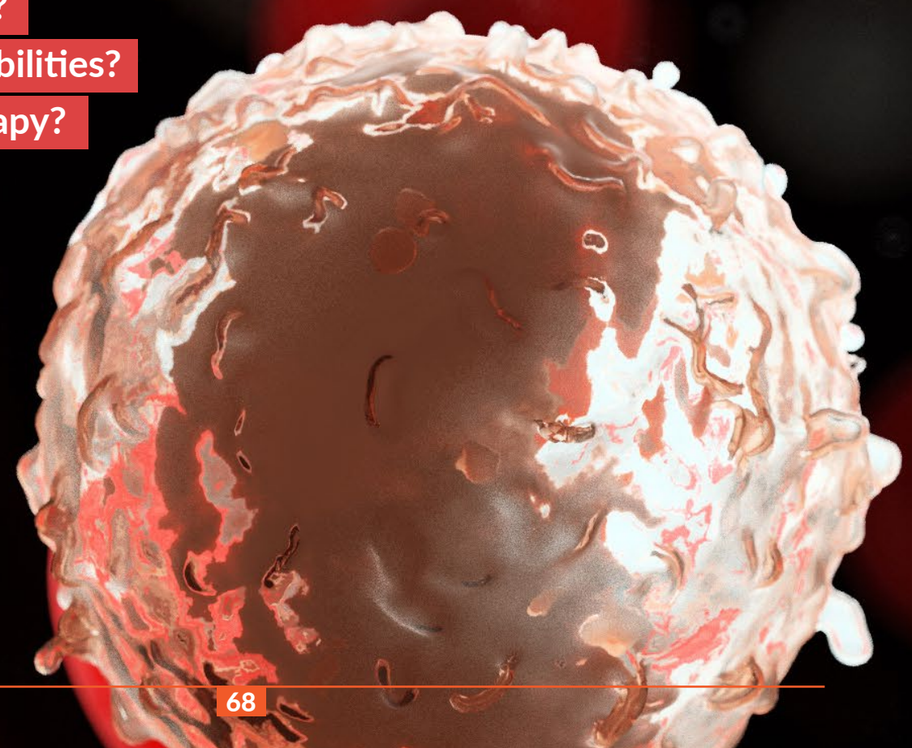
What is leukaemia?
What are the needs?
How to find vulnerabilities?
How to tailor a therapy?



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Acute myeloid leukaemia: an unmet medical need.

Acute myeloid leukaemias (AML) include a greatly heterogeneous group of haematologic malignancies that affects about 3–4 per 100,000 individuals yearly, resulting in 15–20,000 newly diagnosed patients each year in Europe. AML most commonly affects elderly people, with a median age that has reached 70 years. Thus, the incidence of the disease will rapidly rise due to the proportional increase of the ageing population.

In the era of 'precision medicine', the genetic landscape of AML has been defined. Different novel targeted drugs have been developed that are improving the outcomes of patients and are paving the way for more specific drug combinations, possibly less toxic than conventional chemotherapy. Indeed, nine novel therapeutic agents were approved for AML by the Food and Drug Administration (FDA) in the USA since 2017. Nevertheless, for many AML subtypes, a therapy directed toward the genetic lesion causing the disease is not available. Therefore, novel strategies and approaches for drug discovery and development are urgently needed.

The AML with *nucleophosmin (NPM1)* gene mutations: is it targetable?

In AML, the most frequent 'disease-defining' genetic lesion is *nucleophosmin (NPM1)* gene mutations, discovered in 2005 at the Haematology Institute of Perugia University (Perugia, Italy) where Dr Maria Paola Martelli (M.D., PhD) is Associate Professor in Haematology. Since its discovery, Dr Martelli focused her research on *NPM1*-mutated AML and became progressively more convinced that to find a cure for AML concentration needs to be focused on the specific genetic subtypes, each with its own peculiarities. *NPM1*-mutated AML is recognised as a distinct entity in the 2017 World Health Organization (WHO) classification and accounts for about one-third of all AML.

It is peculiar that this leukaemia has been discovered by immunohistochemistry on bone marrow biopsies of patients, which revealed an aberrant cytoplasmic positivity for NPM1 protein instead of the nuclear staining pattern, typical of all the other cells (Figure 1). In physiological conditions, NPM1 wild-type—a protein essential for life—is mainly found in the nucleolus; but it also shuttles between the nucleus and the cytoplasm as a chaperone for various molecules and is involved in ribosome biogenesis and many other important cell functions. Importantly, mutations of *NPM1* with cytoplasmic positivity are uniquely found in AML, making NPM1 mutant protein an ideal target for therapy. However, since NPM1 is not an obviously directly 'druggable' protein, search for the eventual non-oncogene addictions of this disease represents an option to find alternative therapeutic targets.

ContraNPM1AML design

The ContraNPM1AML project aims to dissect and hit the therapeutic targets in AML with *NPM1* gene mutations, hence the acronym of the project. The key challenges are to unravel its unique vulnerabilities and tailor a therapy.

Considering that the main functional consequence of the gene mutation is the production of a mutant protein residing in the cytoplasm that also delocalises the wild-type by interacting with it, the hypothesis under ContraNPM1AML design is that this new condition may alter different equilibria inside the cell and establish a new complex network of possibly unique pathways, responsible for leukaemia, most of which are still unknown. The idea of the project is to focus on the specific 'X' factor or factors making the difference on which leukaemia cells rely. The goal is to identify and target it (Figure 1).

Thus, with the idea that there is something unique to unravel in this disease, Dr Martelli designed a strategy based on two complementary approaches, each focused on either the target or the drug. The hypothesis-driven approach mainly aims to re-think and re-explore the 'known' in the light of the genetic lesion. This requires a deep knowledge of the disease and stems from previous and preliminary observations from the group coordinated by Dr Martelli in the past years.

However, given the complexity of the intracellular pathways and interactions in leukaemic cells, Dr Martelli chose to use wide screening-based approaches with the application of novel technologies and bioinformatics tools for complementary and more comprehensive analyses—aimed at the 'unknown'—to identify new therapeutic targets specific for the disease.

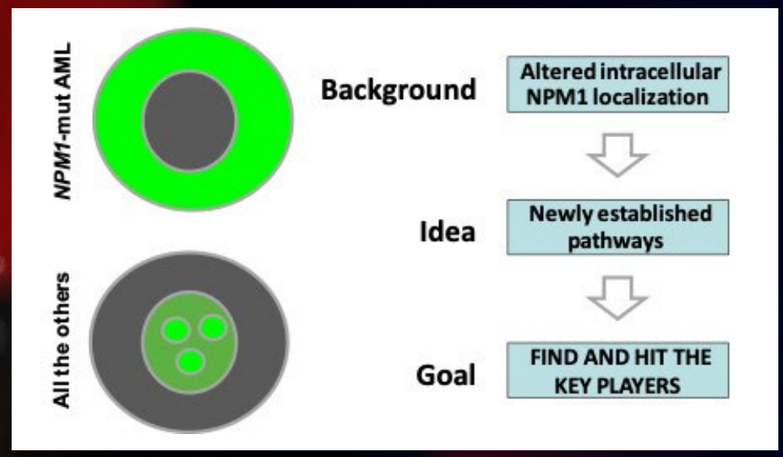

Figure 1: The ContraNPM1AML process to identify and target factors that leukaemia cells rely on.

This part of the project is certainly at higher risk but required due to the complexity of NPM1 as a multitasking protein and the newly established intracellular pathways in the leukaemic cells.

Strategy

Hypothesis-driven approach
Rethinking the 'known' in the light of *NPM1* mutation

Screening-based approach
Applying cutting-edge technologies for the 'unknown'.

On the way to new therapeutic approaches

This research project aims to better understand the leukaemic development process in *NPM1*-mutated AML subtype and identify its Achille's heel and its counterpart, specifically drugs that only kill or more selectively kill AML cells with *NPM1* gene mutations. The final aim is to design and develop clinical trials to prove the safety and efficacy of promising new treatments and make them readily available to patients.

Aims

- **Clarify mechanisms of leukaemogenesis**
- **Unmask novel targets**
- **Drug repurposing**
- **Drug discovery**
- **Design novel 'tailored' therapies**
- **Direct benefit to patients**

ContraNPM1AML is an ambitious project dealing with a challenging leukaemia, but that is giving results and opening new avenues in this field of research.

As NPM1 is a protein involved in multiple functions, it is conceivable that points of vulnerability can be found at different levels. Some of these points were investigated by the team sub-group with the greatest experience on the biology of this disease and have proven to be valid targets.

The implementation of ContraNPM1AML project is providing evidence of peculiar activity against *NPM1*-mutated AML resulting from a series of drugs approved for other diseases. Therefore, these drugs have been 'repurposed' within the project in the setting of *NPM1*-mutated AML in new clinical trials.

Dactinomycin is one of these drugs. Dactinomycin is one of the oldest chemotherapeutic drugs used to treat various tumours, but never reported in AML before, and known as one of the most potent cell stress inductors. Deep investigations on the effects of dactinomycin in cells carrying *NPM1* mutations lead to the demonstration that expression of NPM1 mutant lowers the threshold for stress-induced cell death (Gionfriddo *et al.*, 2021), providing an explanation for its clinical activity in *NPM1*-mutated AML, reported by the same group of researchers. Moreover, with the collaboration of the group coordinated by Prof. Hugues de Thè (Collège de France, INSERM, Paris), it was found that NPM1 mutant impairs vital cellular functions, specifically mitochondrial fitness and function of the PML tumour suppressor, favouring the action of dactinomycin. Interestingly, dactinomycin was found to synergise with venetoclax, a small molecule inhibitor of the anti-apoptotic protein Bcl-2 and one of the latest FDA-approved drugs in AML (Wu HC *et al.*, in press). With these results, we propose dactinomycin as a potential therapeutic alternative in patients affected by *NPM1*-mutated AML with refractory or relapsed disease, deserving further investigation in larger clinical studies and in combinatorial settings.

Aimed at targeting levels of proteins relevant for leukaemia cell survival, Dr Martelli studied the role of omacetaxine mepesuccinate (HHT), a drug that inhibits protein synthesis, in the setting of *NPM1*-mutated AML and found that HHT, besides other relevant players in leukaemia (e.g. anti-apoptotic protein Mcl-1), decreases levels of NPM1 mutant oncoprotein and induces leukaemia cell differentiation. Strikingly, the combination of HHT with venetoclax potently reduced AML tumour burden in mice transplanted with *NPM1*-mutated AML from patients and, therefore, prolonged mice survival. These findings prompted Dr Martelli to translate her discovery from bench to bedside by designing a tailored therapy based on the combination of these two drugs. She thus developed the first pilot clinical trial which was funded in a 5-years project by the Italian Association for Cancer Research (AIRC), and that now is recruiting patients with relapsed/refractory *NPM1*-mutated AML (SynVen-AML, EudraCT number: 2019-001821-29).

Other drugs targeting different pathways have been selected and investigated and are now in the preclinical phase of the studies and show promising results with the potential to be translated in the clinics.

In ContraNPM1AML, Dr Martelli also uses drugs targeting specific pathways or oncogenes as a tool to understand mechanisms of leukaemogenesis or leukaemia maintenance. In particular, she is studying small molecule inhibitors of FLT3 or IDH1/2, as genes frequently co-mutated in *NPM1*-mutated AML and with a cooperative role that still remains elusive. By decoupling signalling derived by the concomitant mutation and *NPM1* expression, terminal cell differentiation of leukaemic cells occurred whilst the 'self-renewal gene signature' associated with *NPM1* mutation persisted, unveiling in vivo, in patients, specific roles for each mutation (Sabino *et al.*, 2020).

Substantial effort was put into two main tasks to discover new targets by identifying essential interactions by high throughput drug library screens and genome-wide interference in *NPM1*-mutated AML. Dr Martelli's team received support from the Horizon 2020-funded CORBEL project in carrying out the highly complex drug screening. The resulting connection with two German research infrastructures, EU-OPENSCREEN and Euro-BioImaging, enabled access to technologies unavailable in Martelli's lab and enabled the screening of large compound libraries to be scaled up. Despite the COVID-19 pandemic, a lockdown, and constantly evolving travel restrictions that slowed cross-border cooperation, the ERC/CORBEL team succeeded in completing the screening, leading to the identification of two classes of drugs highly effective in *NPM1*-mutated AML cell models that are being investigated further in a preclinical setting.

Considerations on ContraNPM1AML and beyond

From the intersection of the genomic and chemical screens, the integration and synergy of the different work packages of ContraNPM1AML, and the planned strategy, expected and effective results include clarifying the mechanisms of leukaemogenesis, unmasking novel targets opening new avenues in the field of drug discovery and designing novel tailored therapies, with the hope of a final direct benefit to patients.

The ultimate goal of this project poses a considerable challenge, but Dr Martelli believes that with her previous and recent discoveries, this goal can be soon reached.

This ERC grant gave Dr Martelli and her team tremendous support in executing a high-gain/high-risk project. It helped the consolidation of her research programme and research group at the haematology department of Perugia University.

The team is currently composed of 11 scientists, divided into three subgroups: the high throughput screening and drug combination unit; the genome-wide interfering screening and modelling unit; and the leukaemogenesis unit. All subgroups are strictly interconnected and flanked by a bioinformatician unit. The team members are also involved in evaluating and characterising drug effects in patients during clinical trials.

During the project, the supervision of Prof. Martelli, and the academic environment gave opportunities for student formation, scholarships and PhD fellowships within this project.

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UNIVERSITÀ DEGLI STUDI DI PERUGIA

PROJECT SUMMARY

Acute myeloid leukaemia (AML) is a group of haematologic malignancies that have been traditionally difficult to classify and treat. Nucleophosmin *(NPM1)* mutations are the most frequent genetic alteration (about 30 per cent) in AML and *NPM1*-mutated AML is a new entity in the WHO classification of myeloid neoplasms. However, mechanisms of leukemogenesis and a specific therapy for this leukaemia are missing. The ContraNPM1AML project aims to unravel the complex network of molecular interactions that take place in this distinct genetic subtype and find their vulnerabilities to identify new targets for therapy. The expected discoveries will lead to novel therapeutic approaches and make clinical trials available to patients.

PROJECT LEAD

Maria Paola Martelli gained her PhD in Biotechnologies in Human Bone Marrow Transplantation, Hematology at the University of Perugia, Italy, where she currently is the Associate Professor in Hematology. Her main expertise and interests include care and treatment of patients with malignant haematological diseases; coinvestigator in clinical trials; biochemistry of tumour-specific proteins (i.e. NPM1, EML4/ALK, BRAF); genomics in onco-haematology; target therapy in acute myeloid leukaemia (AML); and clinical and translational research in AML.

PROJECT PARTNERS

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