Paths to precise prescriptions

Dr Jatinder Lamba explains her current research into individual analyses for acute myeloid leukaemia patients, based on pharmacogenomic pathways, and her hopes for clinical application of the results.

What are the aims of your current research project?

Our goal is to elucidate the molecular mechanisms underlying variability in response to ara-C treatment, to tailor treatment according to patient’s genetic profile and thus achieve maximum therapeutic efficacy while reducing therapy-related toxicity.

With acute myeloid leukaemia (AML) ranked the second most common paediatric leukaemia, what is inhibiting the development of successful treatments?

The two most common AML classification systems use morphology, cytogenetic abnormalities and mutations for stratification but they are ineffective in supporting accurate prognostic or therapeutic decisions. Patient responses to treatments vary even within the same classification, and only a subset of patients have chromosomal abnormalities, mutations or altered gene expression. Thus, the biggest challenge in successful AML treatment is the wide variation in response, and the development of drug resistance.

What evidence have you seen that leads you to believe the genetic polymorphisms in key genes in the Cytarabine (ara-C) metabolic pathway are the cause of variability in treatment response among AML patients?

Ara-C is a prodrug activated by multiple phosphorylation steps to form active metabolite ara-CTP. Incorporating ara-CTP into DNA, instead of deoxycytidine triphosphate, results in chain termination, which blocks DNA and RNA synthesis and causes leukaemic cell death. Thus intracellular levels of ara-CTP are crucial and can influence its therapeutic efficacy. Previous laboratory studies have shown that the intracellular concentrations of ara-CTP are higher in ara-C sensitive cells.

We have observed 40- to 100-fold variation in leukaemic cell intracellular ara-CTP levels in paediatric patients. Additionally our data has shown that cellular sensitivity or resistance to ara-C also demonstrates wide inter-patient variation. Thus single nucleotide polymorphisms (SNPs) or small insertions or deletions in genes involved in ara-C activation or downstream pathways could influence their expression and/or activity which in turn can alter metabolic activation of ara-C. Thus, variations in ara-CTP levels and sensitivity to ara-C could be partly attributable to variations in expression or activity of proteins that affect ara-C uptake, activation and degradation. Collectively these can influence treatment response.

We have demonstrated that the presence of genetic polymorphisms in genes within this pathway is associated with gene expression or enzyme activity and thus with ara-C chemosensitivity in HapMap Cell lines as well as in AML patients treated with ara-C-based chemotherapy.

How far along in your study are you and have you revealed any notable findings to date?

We have evaluated genes involved in metabolic activation of ara-C for inherited genetic variation and analysed their association with multiple pharmacological and clinical endpoints in leukaemic cells obtained from AML patients at various stages in their treatment.

We have published data on significant genetic polymorphisms in genes within this pathway associated with gene expression or enzyme activity and thus with ara-C chemosensitivity in HapMap Cell lines as well as in AML patients. An evaluation of other genes is currently being prepared for publication. In fact in AML patients we have comprehensively evaluated potentially significant genetic variants in ara-C pathway genes and are preparing this work for publication.

In addition to genes involved in activation of ara-C, genes involved in downstream pathways such as the apoptotic pathway are critical for the antileukaemic effect of ara-C. We have found that polymorphisms in the FKBP5 gene are associated with leukaemic blast ara-C chemosensitivity as well as survival in paediatric patients. FKBP5 has no role in ara-C activation but is involved in the apoptotic pathway and is implicated in ara-C chemosensitivity.

My collaborator Dr Stanley Pounds recently developed a method which identifies gene expression signatures and conducts simultaneous analysis of multiple clinical and pharmacological end points in AML patients. Using this method, we identified gene expression signatures associated with detrimental or beneficial therapeutic patterns. We hope to use these signatures to identify patients likely to respond better than others and for development of novel therapeutic agents.

What will be your next step?

Integration of pharmacogenetic markers with disease risk classifications would help in the development of tailored treatment, which would be a major advancement over the current stratified approach.

We intend to translate our information to ease transition to clinical application. We hope to build an algorithm for integrating pharmacogenetic and genetic information as independent prognostic markers into risk classification models to increase the accuracy of forecasting therapeutic outcomes. We are also evaluating epigenetic signatures. Identification of such predictive markers of response helps us to classify patients more accurately and tailor therapies to them.
ACUTE MYELOID LEUKAEMIA (AML) is a blood and bone marrow cancer that affects children and adults alike, but has the worst prognosis of all major childhood cancers, accounting for more than one third of deaths from leukaemia in children. The five year survival rates for AML are about 65 per cent for children under 15 and 5 per cent for patients over 65 years old. In addition, the disease recurs in about a third of children, despite chemotherapy and stem cell transplant treatment for the primary cancer. Most of the improvements in patients’ responses to treatment within the last 40 years have merely arisen from inclusion in clinical trials, improved standards of care and intensive chemotherapy.

Cytarabine (ara-C) has been the core of AML chemotherapy for decades and is one of the key drugs that induce remission. AML chemotherapy includes an induction I regimen primarily comprised of cytarabine and daunorubicin followed by subsequent chemotherapies being conventionally decided according to a system in which patients are grouped into subtypes. The current classification rests on chromosomal abnormalities and gene mutations and is often limited in providing accurate prognostic and/or therapeutic classification. Although the current system of classification is deemed widely acceptable, it is unable to predict accurately the course of disease within risk groups, and there is wide inter-patient variation in response within the same subgroup indicating that the individual patient’s genetic profile might be contributing to the observed response. Moreover, it is clear that identification of predictive genetic and more detailed classification markers is needed for tailoring therapy to achieve maximum benefit. In fact, development of resistance is one of the biggest challenges faced by clinicians and patient healthcare to be critical for the success of a patient-orientated pharmacogenetic study such as hers: “Our study team consisted of me, with 15 years’ experience in pharmacogenomics and designing studies, Dr Stanley Pounds from St Jude Children’s Research Hospital in Memphis, with expertise in guiding statistical analyses and developing novel methods for integrated genomic analysis, and clinical leaders Drs Jeffrey Rubnitz and Raul Ribeiro, who both have extensive experience in treatment of AML and have been involved in multiple clinical trials”.

Advancements in the field of genomics mean that the approach of ‘one size fits all’ is a thing of past.

INVESTIGATIVE TEAM

Lamba is very actively involved in a wider initiative as a Director of the PUMA-IPM, a newly established Institute at the University of Minnesota: “The College of Pharmacy has recently set up an Institute of Individualised Medicine with a mission to advance personalised medicine, based on pharmacogenomic and genomic principles, to achieve maximum benefit for patients”.

Lamba’s first goal was to identify and characterise the genetic variations in key genes involved in the metabolism of ara-C. This would provide an opportunity to identify patients at increased risk of adverse reaction or inferior response to ara-C treatment based on their genetic profile, and help to create opportunities for developing tailored therapy to achieve maximum benefit.

As the field of pharmacogenomics moves towards clinical trials, Lamba hopes to see more healthcare professionals (pharmacists, nurses and clinicians) and genetic counsellors involved in investigations.

DISCOVERY AND TRANSLATION

Lamba’s latest study has now confirmed her hypothesis that genetic polymorphism in the ara-C metabolic pathway is responsible for variations in AML patients’ responses to treatment and the ways in which the toxicity of ara-C based drugs affect them.

The discovery phase of an investigation in Lamba’s laboratory centres on identifying and characterising single nucleotide polymorphisms (SNPs). Lamba and her team identified the genetic polymorphisms associated with enzyme activity in selected ara-C metabolic pathway genes and the regulatory polymorphisms associated with messenger ribonucleic acid expression in these genes. They then determined the association of functionally significant polymorphisms in the genes with phenotypes such as gene expression levels and drug sensitivity.

A key determinant of prognosis for AML patients is race: “SNPs and haplotypes occur with varying frequency in different ethnic groups,” says Lamba. “In fact, some SNPs only occur in specific ethnic groups and are completely absent or very rare in others.”

The functional genetic polymorphisms that were identified in the discovery phase were then analysed in the next phase of the study, the translational phase (which is focused on AML patient cohorts), to discover correlations between them and the responses of patients treated with ara-C based chemotherapy. Lamba has also used genome-wide gene expression signatures to identify predictive genetic markers associated with response, and for this the team used a statistical analysis tool called PROMISE (Projection into most Interesting Statistical Evidence), developed by Dr Pounds.

TECHNOLOGY

As ethnicity plays an integral role in patients’ responses and the outcomes of treatment for black or Hispanic children with AML are significantly poorer than those for white children, Lamba used
INTELLIGENCE
PHARMACOGENETICS OF ARA-C METABOLIC PATHWAY

OBJECTIVES
AML is very heterogeneous and is difficult to treat. The objective of this study is to enhance development of effective treatment strategies for the disease by the integration of predictive pharmacogenetic/genetic, epigenetic and other prognostic biomarkers allowing tailoring therapy to reduce drug toxicity without compromising efficacy. It is hoped that results of the study will help in the development of personalised treatment approaches, which will be a major advancement over current strategies.

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FUTURE DIRECTIONS
Ara-C belongs to the nucleoside analogue class of drugs, which impede DNA and RNA synthesis in cells. Lamba feels that the results of her investigations would be applicable to other nucleoside analogue drugs for diseases other than AML as they share the same metabolic pathway. “The majority of other nucleoside analogues are currently used in treatment of haematological malignancies, but one (gemcitabine) is used as a chemotherapeutic agent in treatment of solid tumours, primarily pancreatic, lung and ovarian,” she elucidates. Moreover, certain nucleoside analogue drugs that are used to counter HIV are activated by some of the same genes as in the ara-C pathway.

Lamba is hopeful that in future she will be able to test her findings in a clinical trial with patients randomised in two groups – one group receiving the current standard of care and the other receiving treatment tailored according to predictions of their likely responses to ara-C, based on their genetic signatures and variations. She is sure that her methodology will be validated by comparisons of the groups’ responses to treatment and that her work will ultimately lead to better treatment strategies and hence better quality of life for AML patients.