neoplasia. Reversal of abnormalities in DNA methylation may restore expression of genes with tumor-suppressive function and provide a novel approach to cancer therapy. DNMT inhibitor azacitidine has been the first FDA approved agent for treatment of myelodysplastic syndromes.
For high risk MDS (IPSS Int-2 and more) the therapeutic goal is to retard AML transformation and improve cytopenia. Given the epigenetic alterations marking disease progression, HR-MDS are ideal candidates for therapy with DNMT inhibitors. Several studies have demonstrated that subcutaneous azacitidine determines sustained hematologic improvement together with amelioration in quality of life and delay in AML transformation in a high percentage of MDS patients. Recent re-evaluation of the results of all azacitidine trials, with the application of the revised IWG criteria of response to treatment, has confirmed the efficacy of the drug, indicating a 10–17% CR rate and validating an overall response of 44–47%. In all the studies, achievement of response was obtained after a median of 3 cycles, with 75% and 90% of responders achieving a response by 4 and 6 cycles of therapy respectively. A randomized phase III trial (supportive care vs azacitidine 75 mg/m$^2$/day for 7 days every 4 weeks) is near to conclusion and will evaluate response and survival advantage in HR-MDS patients treated with this agent. The use of azacitidine has been recently considered even in the set of bone marrow transplantation, both as preparatory regimen or as maintenance after BMT.

## 11 Combination epigenetic therapy

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The understanding of epigenetic alterations in cancer and their in vitro modulation has resulted in the development of new therapeutic approaches for patients with myelodysplastic syndromes and leukemias. It has also become apparent that combining different forms of epigenetic therapy, such as a hypomethylating agent with a histone deacetylase (HDAC) inhibitor, not only results in synergistic reactivation of epigenetically silenced genes, but also in significant antileukemia activity. We have exploited this concept in a number of sequential clinical trials using either 5-aza-2′-deoxycitine or 5-azacytidine in combination with valproic acid and more recently with the HDAC inhibitor MGCD103. In this presentation, we will review the rational for such studies, potential schedules using short courses of high dose HDAC inhibitors in combination and results of ongoing clinical trials at our center, as well as the initial analysis of large scale epigenetic profiles in specific patient populations. These results may help in the development of new clinical trials of epigenetic therapy in human cancer.

## 12 Treatment of myelodysplastic syndromes with azacitidine and thalidomide

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The demethylating agent 5-azacitidine induces responses in 48% patients with all subtypes of MDS and significantly delays median time to AML transformation or death (12 v 21 mths p = 0.007) [1]. Thalidomide, likely working through a number of immunomodulatory, anti-angiogenic and anti-cytokine mechanisms has also shown promising results with 11–56% (by intention to treat) MDS patients obtaining a response, though with poor tolerance at doses above 100 mg/day[2]. Clearly not all patients respond to these drugs as single agent, and given the heterogeneous nature of MDS, differing mechanisms of action of these agents and their differing side effect profiles, combination therapy with 5-azacitidine and thalidomide in MDS may be tolerable and in fact improve overall response rates. A small pilot study of 29 patients with MDS or AML treated with this combination (5-azacitidine 75 mg/m$^2$/d for 5 days every 28 days and thalidomide continuously 50–100 mg/d) was reported by Westervelt et al at ASCO 2006 [3]. They reported good tolerance of the combination with 6 of 25 evaluable patients obtaining a complete remission and 14 (56%) a haematologic improvement. The Australasian Leukaemia and Lymphoma Group (ALLG) has commenced a Phase I/II, single arm, open label, multi-centre trial to determine safety and efficacy of combination therapy of 5-azacitidine (Vidaza) and thalidomide in patients with MDS. The primary objective is safety and tolerability of the combination, secondary objectives detailing response rates (per modified IWG criteria [4]) and duration, QOL parameters (as measured by EORTC QLQ-C30 and FACT-general) and biomarkers. Eighty patients with MDS will be enrolled, commencing April 2007, from around Australia and New Zealand and responses compared to published results of single agent 5-azacitidine. In addition to Best Supportive Care, all patients will be treated with 5-azacitidine subcutaneously 75 mg/m$^2$/d for 7 days every 28 days and thalidomide commencing at 50 mg/d and increasing to a maximum of 100 mg/day orally (up to 12 months but no longer due to the risk of peripheral neuropathy). Patients will stay on 5-azacitidine until either prohibitive toxicity or progressive disease with dose delays and modifications outlined according to toxicity, predominantly haematologic. Changes in methylation of p15$^{INK4B}$, a commonly hypermethylated gene in MDS, and other genes including CDH1, HIC1 and SOCS1 will be measured utilising a newly developed semi-quantitative technique of methylation specific high resolution melting (MS-HRM) of amplified, bisulphate treated DNA isolated from magnetic sorted CD34+ bone

marrow cells. Methylation changes will be correlated with clinical responses and potential predictive markers explored. In addition, a number of immunologic and apoptotic assays will aim to further our knowledge of mechanisms of action particularly of thalidomide including T/NK cell enumeration and activation and serum cytokine changes by means of a multiplex cytokine bead array.

## References

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## 13 Treatment of acute myeloid leukemia (AML) and high-risk myelodysplastic syndromes (MDS) in the elderly with azacitidine and gemtuzumab ozogamicin (GO)

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AML and high-risk MDS in patients over the age of 60 carry a poor prognosis. With standard chemotherapy, about 46% achieve a complete remission (CR) and treatment-related mortality approaches 30%. In 2005, we began a pilot trial for elderly patients with newly diagnosed AML or high-risk MDS, using hydroxyurea to lower the white cell count followed by azacitidine and GO. This combination was chosen for its non-overlapping mechanisms of action, low toxicity and ability to deliver treatment in the outpatient setting. There are several reasons to believe that the proposed sequence of treatment may be beneficial: (1) Azacitidine may increase CD33 expression and decrease p-glycoprotein expression, (2) Degree of CD33 positivity is associated with response to GO, (3) P-glycoprotein plays a significant role in resistance to GO therapy, (4) Lowering of WBC count increases uptake of GO by marrow blasts, thus allowing use of a lower dose of GO. The treatment regimen is as follows: If initial WBC count was >10,000/ul, patient was started on hydroxyurea 1500 mg orally twice daily. Once WBC count was <10,000/ul, the patient received azacitidine 75 mg/m$^2$ s/cu daily for 7 days. On D8, GO 3 mg/m$^2$ iv infusion was administered. A bone marrow was performed on D14 and the induction therapy was repeated if patient had residual disease. Those who achieved a CR went on to receive one consolidation treatment with azacitidine and GO in the same doses. Subsequent treatment was left to the discretion of the treating physicians. A total of 15 patients have been treated so far. Median age was 77 (62–83) and 7 were males. Two had RAEB and 13 AML. Four patients with AML had previous history of MDS. Nine had cytogenetic abnormalities at presentation. Eleven (73%) achieved a CR. Eight patients developed neutropenic fever including 1 typhlitis, all requiring hospitalization. There were no treatment–related deaths during induction therapy. Median survival is 10 months (1–20 mo) and 7 remain alive at this time. The trial is ongoing with an accrual goal of 20 patients. Two patients with relapsed AML were treated with this regimen off protocol. Both achieved CR, one lasting 11 months, the other >12 months. Thus, cytoreduction with hydroxyurea and leukapheresis followed by azacitidine and GO therapy appears to be a safe and effective regimen for elderly patients with AML and high-risk MDS. These preliminary results need to be confirmed in a larger Phase II study.

## 14 Cytogenetics of myelodysplastic syndromes and detection of del 5q

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Over 50% of all myelodysplastic syndromes (MDS) patients have cytogenetic abnormalities at the time of diagnosis [1,2]. These include a wide array of cytogenetic changes, such as monosomy, partial deletions and insertions of chromosomes, and trisomies. The type and complexity of cytogenetic abnormality closely relates to survival and the risk of progression to acute myeloid leukaemia (AML) [2]. Partial or complete deletion of the long arm of chromosome 5 (called deletion 5q; del 5q) is the most common cytogenetic abnormality found in MDS patients, occurring in 10–15% of all *de novo* MDS patients [1,2]. Prognosis of MDS patients with del 5q worsens with additional cytogenetic changes and increases in blast percentages [3,4]. Therefore, in the subgroup of del 5q patients with the 5q– syndrome (who have isolated del 5q and a blast percentage below 5%), prognosis is better than for other patients with del 5q [3,4]. Newer methods of cytogenetic testing in MDS patients, besides conventional metaphase karyotyping, are rapidly evolving. These methods include, among others, fluorescent *in situ* hybridization (FISH), chromosome painting (multicolour FISH and spectral karyotyping (SKY)), whole chromosome painting (WCP), loss of heterozygosity (LOH), comparative genomic hybridization (CGH), and gene expression analysis by the Affymetrix array. All these methods have their own pros and cons in clinical practice, and understanding these is important in optimal clinical decision making. Lenalidomide has shown to be highly effective in transfusion-dependent Low-/Int-1 IPSS risk MDS patients with del 5q in particular [5]. Newer cytogenetic detection techniques can detect del 5q more easily and accurately, and may therefore be very useful in the selection of patients for lenalidomide treatment, as well as prediction of response to lenalidomide [6]. In conclusion, understanding cytogenetics in MDS and the techniques to detect abnormalities such as del 5q accurately and easily, is