International Workshop on CLL, Houston, Texas, October 2011

This is an overview of some of the areas I found interesting, and is by no means comprehensive, as there were many excellent presentations for which I don't have room to include! Rather than try to cover all areas, I have focussed on a few presentations and covered them in depth. For readers wishing to know more, abstracts from the meeting have been published in *Clin Lymphoma, Myeloma & Leukemia*, Supplement 2, October 2011 .

NOTCH1

Jonathan Strefford and colleagues investigated the frequency and significance of *NOTCH1* mutations, in patients who were on the CLL4 trial and also in a cohort of stage A patients. Previous studies had identified a frequency of 10% mutations in CLL at diagnosis, but this increased to 31% for patients progressing to Richter transformation, and 21% for chemorefractory patients. Furthermore, *NOTCH1* mutations were an independent prognostic marker.

To investigate this further, the current study used cases from the CLL4 trial (n= 468) and a separate cohort of patients at diagnosis (n= 399). They found a frequency of 3% *NOTCH1* mutations in cases at presentation, but this increased to 8% in cases from CLL4. Mutations were associated with high CD38 and ZAP70 ($p<0.001$) and reduced OS, and shorter time to treatment. Constitutive Notch signalling is known to contribute to drug resistance in CLL (*via* regulation of *NF-κB* target gene expression, and AKT), so the obvious question is whether it would be possible to target Notch. Unfortunately, Notch receptor inhibition is associated with complications and off-target effects, and therefore it is not clear whether this will be an effective therapeutic strategy. Importantly, *NOTCH1* mutations are mutually exclusive with *TP53* mutation, indicating that they probably represent distinct mechanisms that contribute to chemoresistance.

PI3K δ inhibitor, CAL-101

Some of the most exciting new therapeutic approaches are exploiting the importance of the B-cell receptor in driving pro-survival signals. One such agent is CAL-101 (now GS-1101), and **Jennifer Brown** and colleagues (Dana Faber, Boston) presented data from a recent clinical trial which examined patterns of nodal response and lymphocytosis in receiving CAL-101.

Previous *in vitro* studies have shown CAL-101 to be a selective and potent inhibitor of PI-3 kinase δ, resulting in apoptosis in CLL (and other haematological malignancies where this isoform of PI3K is highly expressed) by inhibiting signalling to pro-survival pathways. Recent studies have shown that CAL-101 has efficacy in a phase I setting and can induce a durable response even in heavily-treated patients. This trial aimed to compare the efficacy of CAL-101 alone compared to its effects in combination with either Rituxumab or bendamustine, in a group of patients who had received up to 5 prior therapies.

Treatment was relatively well-tolerated with few cases of Grade 3 or higher toxicity, although most of these occurred in the single agent arm. Most patients experience a significant reduction in node size which resulted in concomitant increase in the number of

lymphocytes in the peripheral blood. This meant that successful treatment led to a transient and dramatic increase in the ALC, due to a redistribution of lymphocytes. This observation supports the proposed mechanism of action of CAL-101, in reducing the survival signals in the lymph node micro-environment, releasing cells into the peripheral blood. Interestingly, co-administration of rituximab decreased this effect, and co-administration with bendamustine ablated it. In terms of outcome, the combination arms were more effective at increasing PFS but the outcome data had to take into account the transient lymphocytosis, which in this context is indicative of proof-of-mechanism. Further demonstration of mechanism was the reduction in AKT phosphorylation and in CXCL13 signalling, which is known to regulate homing of CLL cells in the bone marrow. It is inferred that release of cells from the lymph node makes them more susceptible to targeting by rituximab and bendamustine.

This is an exciting new therapeutic approach, and it will be interesting to follow progression of agents which interfere with B-cell receptor signalling (see also Btk inhibitor, below), particularly as these agents may also have efficacy in those patients that we find most difficult to treat with current chemo-immunotherapies like FCR. Perhaps the most challenging area will be to determine if such combination therapies can give long-term responses and can be well-tolerated by elderly patients.

Bruton's tyrosine kinase (Btk) Inhibitor, PCI-32765

Shih-Shih Chen, in a study led by Jan Burger & Nicholas Chiorazzi 's groups, won a prize for her excellent presentation and work on the Btk Inhibitor, PCI-32765, another agent that targets the functions of the B-cell receptor. BTK is critical for lymphocyte function and trafficking, mediated by the B cell receptor (BCR) and chemokine receptor CXCR4. PCI-32765 has been shown to be effective in decreasing lymph node size, with transient lymphocytosis followed by decrease in ALC, a similar scenario to the effects of CAL-101.

Shih-Shih used the EμTCL1 mouse model to investigate the mechanism of action of PCI-32765, and found that up to 4 weeks after being injected with TCL1 cells, mice had a transient lymphocytosis following treatment, and significantly reduced lymph nodes. Control mice had typical CLL symptoms including weight loss, lethergy, hepatosplenomegaly and lymphadenopathy. Further studies showed that Phospholipase Cγ2 (PLCγ2 -a downstream target of BTK) levels were reduced in treated mice and that chemokine-controlled migration, (*via* CXCL12 or its receptor CXCR4) was lower, indicating that homing of CLL cells was being blocked by PCI-32765. It was proposed that the smaller spleen/lymph node observed in treated mice was due to PCI-32765-promoted migration of cells out of lymph, with a block of return of cells from the peripheral blood. Shih-Shih also examined proliferation using BrdUrd staining, and found deceased BrdUrd incorporation in spleen and lymph of treated mice. In summary, targeting BTK (and its receptors/downstream targets) with PCI-32765 delays CLL progression *via* re-expressed CXCR4 and reduced proliferation, due to decreased trophic stimuli from the BCR or CXCR4 in the microenvironment. (For further information-this study has recently been published in *Blood*.)

Michael Hallek gave an extensive review of treatment, and underlined the improvement in outcome for patients with current immuno-chemotherapies. Michael posed the question of

whether the time is over for single agent chemotherapy**.** However, a recent study (Catovsky, 2011) confirms that Chlorambucil still has a major role to play, producing a good ORR of 60-75% ***when used at the optimum dose*** of 60-70mg/m ^{2} (response was only 30-50% for 40-50mg/m ^{2}). Importantly, in comparison with Fludarabine, there is a similar PFS and OS (data from CLL4) but even at 70mg/m^2, chlorambucil is less toxic than fludarabine or bendamustine, and may be suitable for less fit patients. The other advantage is that patients that do not respond to chlorambucil front-line therapy often DO respond to second line treatments. Current trials (e.g. combination with rituxumab) will address the future role for chlorambucil for CLL treatment.

During the discussion, there was some lively debate about how aggressively patients should be treated- for example is there a real possibility of secondary tumours following FC? These are rare, and the effectiveness of front-line treatment is key, because a successful front-line treatment (e.g. achieving MRD at first attempt) will significantly increase the chances of a longer PFS and good outcome.
Michael described how FCR is now the standard (for patients that can tolerate FCR) and how it leads to higher OS rates- which argues for more aggressive treatment up-front. There is also a need to define high risk patients following poor response to FCR, using MRD data and the presence of del(17p) or unmutated IgVH. It may be that for these high risk patients, lenalidomide may be an option. Variations on FCR are currently being tested, including Bendamustine + R (Fischer K, JCO, 2011) which has a 60% ORR, and 45% ORR in fludarabine-refractory patients (only 7% response in del(17p) cases).

John Byrd reviewed novel treatments that are currently being evaluated. It is clear that CLL is not CML- i.e. CLL is not driven by a specific molecular event that can be targeted. Rather, it is a disease driven by multiple factors and therefore chemo/immunotherapy combinations will remain essential. However, John and Michael Hallek raised the question of whether targeted therapy is now becoming a reality: Are we moving towards being able to stratify patients?

For example, del(11q) patients respond well to FCR (they have a similar outcome to del(13q) patients). ATM-mutated patients may also benefit from PARP inhibitors (phase I trials are in progress). del(17p) patients do not respond to chemotherapy but in recent trial of alemtuzumab + prednisolone (Peter Hillmen), 30% of patients achieved a PR (65% of these were untreated). Lenalidomide may be useful either as front-line therapy for elderly patients, or for those not responding to FCR.

Other antibodies being investigated include Ofatumumab, the second generation anti-CD20 Mab, which may have enhanced cell killing compared to rituximab. Also GA101, a glycoengineered peptide-based humanised type II anti-CD20 antibody may increase direct cell death–inducing capacity with improved Ab-dependent cellular cytotoxicity. Early data suggest that it is more potent than rituximab (at equivalent concentrations) in depleting CLL cells *in vitro* and a phase 1 study with GA101 in heavily pre-treated patients showed promising single-agent activity.

There are also novel CDK inhibitors, following on from flavopiridol, including dinaciclib, a broad-spectrum CDK inhibitor. Also, **Rong Chen** from the MD Anderson showed novel data on the efficacy of SNS-032, a novel inhibitor that seems to target Cdk9 in CLL cells.

Other new agents include Sapacitabine, a DNA strand-breaking nucleoside analogue which is currently undergoing clinical trials for AML. **Bill Plunkett** described the unusual mechanism of action of Sapacitabine, which a prodrug of the cytosine nucleoside analogue, 2′-*C*-cyano-2′-deoxy-1-β-D-*arabino*-pentofuranosyl-cytosine. Sapacitabine stimulates repair *via* the nucleotide excision repair pathway, but also stimulates recombinational repair, with ATM-defective cells being particularly sensitive compared to ATM-functional cells.

In a final discussion of the scientific committee at the end of the meeting, there were a few key areas that were discussed:

- The exciting potential of the agents like CAL-101 and those that target BTK, particularly as these agents are effective on heavily-treated patients and may have efficacy in patients harbouring del(17p).
- There is now a real possibility of targeted treatment, guided by patient stratification based on molecular and cytogenetic abnormalities. Biomarkers can be used for targeted treatments, to show response and proof-of-mechanism.
- MRD measurements are key, and in those laboratories where this is routine, MRD can very sensitively measure response to treatment. This is important as it is clear that achieving a full response initially gives a better long-term outcome.
- Michael Keating highlighted the fact that our shared goal is to improve patient care and treatment, and raised the possibility of a large iwCLL database to facilitate the sharing of data to help us collectively achieve of this aim. We look forward to iwCLL 2013!