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Beyond fludarabine-cyclophosphamide-rituximab (FCR) in CLL
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David Gottlieb is Professor of Haematology at the University of Sydney. He has been actively involved in clinical stem cell transplantation for 25 years, commencing with the early establishment and expansion of the Blood and Marrow Transplant Service at Westmead Hospital. He has a long-standing interest in cellular immunotherapy for the treatment of infection and malignancy in the immunocompromised, specifically in patients undergoing stem cell transplantation and potent chemoimmunotherapy. He is currently focusing on adoptive immunotherapy for opportunistic infection and on ways to optimise the generation of anti-leukaemic T cells that could be used to reduce the incidence of post-transplant disease recurrence.

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This publication is a summary of a recent presentation by Professor David Gottlieb, Professor of Haematology at Westmead Clinical School, Westmead Millennium Institute for Medical Research, Westmead Hospital, University of Sydney, Australia.

He spoke to medical oncologists, haematologists and oncology registrars in Auckland in October 2010 about chronic lymphocytic leukaemia and beyond immunochemotherapy with the use of fludarabine, cyclophosphamide and rituximab.

Early results of a chemoimmunotherapy programme consisting of fludarabine, cyclophosphamide, and rituximab (FCR) showed that it was highly active as initial therapy in previously untreated chronic lymphocytic leukaemia (CLL), resulting in a complete remission (CR) rate of 70%, a nodular partial remission rate of 10%, and a partial remission (PR) rate of 15%, for an overall response rate (ORR) of 95%.[1] The long-term outcome of this regimen for all 300 patients at a median 6-year follow-up was reported recently; the addition of rituximab to FC doubled the CR rate and duration of remission to 72% and 80 months, respectively, in a study involving 300 patients with CLL followed-up for a median of 6 years.[2] In a comparison of FCR with previous generations of front-line fludarabine-based CLL regimens at the same institution, FCR was associated with a significantly superior overall survival (OS); 6-year OS rates for patients receiving fludarabine (F), fludarabine and cyclophosphamide or mitoxantrone (FC/M) were 54%, 59%, and 77%, respectively ($p<0.001$ for FCR vs other regimens). Other 6-year outcomes for the FCR regimen included a response rate of 95%, CR in 72%, nodular PR in 10% and PR in 12%; 5% of patients failed therapy due to disease resistance or early death.

Following on from this study, the German Chronic Lymphocytic Leukaemia Study Group (GCLLSG CLL8) phase 3 trial investigated the effects of rituximab-based front-line chemoimmunotherapy in CLL.[3] The study enrolled treatment-naive, physically fit patients, aged 30–81 years, who had CD20-positive CLL. They were randomised to 6 courses of IV fludarabine (25 mg/m^2/day) and cyclophosphamide (260 mg/m^2/day) for the first 3 days of each 28-day treatment course with (n=408) or without (n=409) rituximab (375 mg/m^2 on day 0 of the first course, and 500 mg/m^2 on day 1 of second to sixth courses). At an extended follow-up of 37.7 months, CR (44.1% vs 21.8%; $p<0.01$) and ORR (95.1% vs 88.4%; $p<0.01$) rates were higher in the FCR group than in the FC group. Stable disease (SD) and progressive disease (PD) rates were 3.9% and 1.0%, respectively, in the FCR group; significantly lower than the corresponding values in the FC group (7.8% and 3.8%). Importantly, those patients who achieved a CR did much better than those who attained a PR or had at-risk disease. Furthermore, progression-free survival (PFS) in the FCR group was prolonged in patients with more favourable prognostic factors; those with disease in Binet stage B had a median PFS of 51.8 months, whereas it was 40.7 months in those with Binet stage C disease. The analysis also showed that OS was significantly improved with the addition of rituximab (OS rate at 37.7 months: 84.1% vs 79.0%; $p=0.01$). These results provide a base for the future direction of chemoimmunotherapy, commented Prof. Gottlieb. There is room for improvement, even in patients with the best response; those with a minimal residual disease (MRD) level of $<10^{-4}$ in peripheral blood at 2 months post-therapy experienced disease progression within 4 to 5 years.

Potential therapeutic possibilities superior to FCR

Future therapeutic possibilities for obtaining and/or maintaining low MRD+ or MRD-negative status and that may prove superior to FCR include:

- Chemotherapy: bendamustine
- CDK inhibitors: flavopiridol
- Monoclonal antibodies: GA101, rituximab, alemtuzumab
- BH3 mimetics: ABT-263
- Immunomodulatory drugs (IMiDs): lenalidomide
- Cell therapy.

While many of these agents are under investigation, their place in the therapeutic armamentarium remains as yet undetermined (i.e. for use in induction, consolidation, or maintenance of MRD).

Chemotherapy

The benefits of bendamustine, an agent that combines alkylating and purine anti-metabolite properties, were demonstrated in a randomised phase III clinical trial in 319 patients (≤75 years of age) with previously untreated advanced CLL, who were randomised to receive a maximum of 6 cycles of treatment with either bendamustine (100 mg/m^2 D1 and 2) or chlorambucil (0.8 mg/kg D1 and 15).[4] The ORR was over twice as high for the bendamustine group compared with the chlorambucil group (68% vs 31%; p<0.0001) and the number of CRs alone was dramatically higher with bendamustine (31% vs 2%). Median PFS was significantly improved with bendamustine compared with chlorambucil (21.6 vs 8.3 months; p<0.0001). However, bendamustine was associated with a higher frequency of grade 3–4 adverse events (AEs) (40% vs 19%).

The use of bendamustine in combination with rituximab was evaluated in the front-line treatment of 117 patients with previously untreated CLL, by the GCLLSG phase 2 study.[5] All patients received up to 6 cycles of bendamustine 90 mg/m^2 D1+2 plus rituximab 375 mg/m^2 C1 and 500 mg/m^2 C2–6. At a median follow-up of 15.4 months, the ORR was 90.9%; CR 32.7%, PR 55.5% and nodular PR 2.7%. The median PFS was not reached at 18 months. Myelosuppression was the major adverse event. An MRD level <10^{-4} was observed after completion of therapy in 29 of 50 evaluable patients in peripheral blood, while 7 of 25 patients achieved <MRD^{-4} in bone marrow. Differences in response were observed among the genetic subgroups: 19 of 21 patients with 11q deletion achieved a remission with 10 PR and 9 CR (ORR 90.5%), while 17 of 19 patients with chromosome 12 trisomy responded (14 PR, 3 CR, ORR 89.5%). In the high-risk group with 17p deletion, only 3 of 7 patients attained a PR (ORR 42.9%), whereas 56 of 63 patients (ORR 88.9%) with unmutated *IGHV* gene status responded. Based on these data, the GCCLSG is now undertaking a randomised phase III trial of bendamustine plus rituximab in comparison to FCR in the first-line treatment of CLL in treatment-naïve patients (CLL10 protocol).

Cyclin-dependent kinase inhibitors

Beneficial agents are being sought for use in the poor prognostic 17p deletion subgroup. The first agent to be investigated has been flavopiridol, a serine/threonine kinase inhibitor that inhibits cyclin-dependent kinases (CDKs) that control cell cycling. Flavopiridol clinical activity varies significantly based upon schedule of administration; 45% PR was achieved with a 30-minute infusion followed by 4 weekly 4-hour infusions, 2 weeks off.[6] In relapsed CLL, flavopiridol was associated with an ORR of 53% (2% CR, 47% PR, 5% nodular PR) including those with 17p deletion and 11q22 deletion, *regardless of node size*, with a PFS lasting 10–12 months.[7]

Nevertheless, while responses are rapid, a well-known consequence is tumour lysis syndrome (TLS), which must be managed aggressively (hyperacute TLS and rise in potassium require urgent dialysis), with also hypotension and diarrhoea. Flavopiridol is also associated with a low incidence of life-threatening opportunistic infections.

Monoclonal antibodies

Antibodies are now considered to be a standard treatment for CLL. Prolonged data from the GCLLSG trial have demonstrated the role played by rituximab in PFS and OS, with the FCR arm achieving significantly better median PFS (51.8 months) and OS values (87.2 months) at 3 years post-randomisation compared with the FC arm (32.8 and 82.5 months, respectively).[8]

The role of rituximab consolidation and maintenance therapy has been investigated in CLL.[9] For induction treatment, 120 patients received daily fludarabine (25 mg/m^2) on days 1–5 at 28-day intervals for a total of 6 cycles. The 16 patients with MRD >1%, 22 with CLL and an absolute lymphocyte count (ALC) >1000 within 1 year and 16 in PR were given consolidation/maintenance with 4-monthly cycles of rituximab at a dose of 375 mg/m^2 followed by 12-monthly doses of rituximab at a dose of 150 mg/m^2. The median follow-up duration was 50 months. All patients experienced a prolonged PFS from the end of induction treatment (40% at 9 years); moreover, consolidation and maintenance therapy significantly prolonged response duration in persistently MRD-negative patients (>1 year) versus MRD+ not consolidated (75% vs 9% at 4 years). These data suggest that rituximab may be a good antibody for maintaining remission in the high-risk subset of MRD-negative cases, stated Prof. Gottlieb.

The *in vitro* activity of the next generation monoclonal antibody, GA101 (a third-generation humanised glyco-engineered IgG1 anti-CD20 antibody), has been examined on B cell depletion/apoptosis in whole blood CLL samples that were genetically characterised with respect to genomic aberrations, TP53 mutation and *IGHV* mutation status, as well as clinical course and immunophenotype.[10] At an equivalent concentration, GA101 resulted in a 5–100-fold greater antibody-dependent cell-mediated cytotoxicity (ADCC) than rituximab. Furthermore, modified elbow hinge sequences within the antibody variable framework regions induced greater apoptosis, resulting in more potent B cell-depleting capacity than rituximab. No obvious differential effect was observed on genetic subgroups including TP53 mutation/17p deletion (n=10).

A phase I dose-escalation study (400–2000 mg D1, 8, 22 then every 3 weeks for a total of 9 infusions) was initiated to determine the safety, tolerability, dose-limiting toxicity (DLT), and pharmacokinetics of GA101 given as a single agent to 13 patients with CD20+ B-CLL who had received a median of 3 prior regimens (including fludarabine and rituximab-containing therapy) and for whom no therapy of higher priority was available.[11] GA101 was well tolerated with no DLTs or dose reductions. The ORR was 62% (8/13) with 1 CR with incomplete haematopoietic recovery (Cri), 7 PR and 5 SD observed across all FcγIIIRA [158F/V polymorphism] genotypes with no clear dose relationship established. Responses were reported as ongoing with durations ranging from 3.5+ to 8+ months. End of treatment MRD from 7/11 evaluable patients was detectable for 6 patients (median reduction of 2 log, range 2–4) and negative for one (despite attaining SD, as assessed by CT scan). Side effects included infusion reactions, transient neutropenia (n=9), thrombocytopenia (n=1) and infections (n=10).

A phase II clinical trial assessed CR and OS in high-risk patients aged <70 years with untreated CLL and beta-2 microglobulin (B2M) levels of ≥4 mg/L given alemtuzumab plus the FCR regimen (CFAR) as front-line therapy.[12] The treatment regimen consisted of F 20 mg/m^2 D3–5, C 200 mg/m^2 D3–5, R 375–500 mg/m^2 D2 and A 30 mg IV D1, 3 and 5 every 28 days for 6 cycles. CR was achieved in 70%, nodular PR in 3%, PR in 18%, and 7% pts did not respond; the ORR was 92%. There was no significant correlation between CR or OR with Rai Stage, IgV_H mutation status, FISH status, ZAP70 and CD38 expression. After a median follow-up of 24 months, 19 (32%) patients had progressive disease. Patients with 17p deletion and unmutated IgV_H had a significantly shorter time to progression (TTP). Grade 3–4 neutropenia and thrombocytopenia occurred in 31% and 13% courses, respectively. Major infections, including pneumonia and sepsis, were reported for 10 (17%) patients. Minor infectious such as bronchitis, urinary tract infections and herpes zoster were reported for 15 (25%) patients. Alemtuzumab-associated infusion reactions occurred in 42 (71%) patients. CMV reactivation occurred in

7 (12%) patients, all of whom were on valacyclovir prophylaxis. There was 1 death due to CMV pneumonia. The median OS for all patients had not been reached at the time of reporting (49+ months) and the median TTP was 38 months.

Alemtuzumab consolidation improved the CR and MRD-negative rates after FR induction in the CALBG 10101 phase II study, in which 101 untreated patients were administered 6 cycles of F+R.[13] Those with stable or responsive disease began alemtuzumab 30 mg SC 3x/week for 6 weeks, 4 months after chemotherapy. Overall, CR and PR rates after FR induction were 90%, 29% and 61%, respectively, and 15% were MRD-negative by flow cytometry. On alemtuzumab, 28 of 45 converted from PR to CR and 3 of 6 MRD+ CR became MRD-negative CR. However, alemtuzumab consolidation was associated with significant toxicity, particularly severe infections in patients who achieved a CR after FR induction. Rates of grade 3–4 neutropenia and thrombocytopenia were 43% and 19%; 5 patients in CR after FR died from infections after alemtuzumab (viral meningitis, *Listeria* meningitis, *Legionella* pneumonia, CMV and PCP pneumonia), and 1 patient in PR after FR died after alemtuzumab of Epstein-Barr virus viraemia. Deaths occurred both during and for up to 7 months after alemtuzumab therapy.

The BH3 mimetic ABT-263

ABT-263, an orally bioavailable BH3 mimetic, induces apoptosis in Bcl-2 overexpressing human lymphoma cell lines and primary CLL cells. A phase 1/2a dose-escalation trial evaluated ABT-263 PK, safety and antitumour activity of two dosing schedules in 29 heavily pretreated patients with relapsed or refractory CLL.[14] A large number of patients had no significant reductions in lymphocytosis; however, 2 patients had radiographically-confirmed partial responses (99% and 79% reductions) and 3 had as yet unconfirmed nodal regression (100%, 71% and 55%). Seven patients maintained a ≥50% decrease in circulating absolute lymphocyte count for ≥2 months with 2 patients having PR by physical examination; the ORR was 33% (excluding 3 patients treated at doses <110 mg). Stable disease was noted in 8 patients and 2 patients had progressive disease. Responses tended to be durable, with the median PFS not yet reached at the time of reporting with a median time on study of 9 months. In an analysis of responses (i.e. including PR, >50% fall in lymphocytes, or both) in 21 patients with cytogenetic data dosed at >100 mg/day, responses were achieved by 5 of 6 patients with 17p deletion, 4 of 5 patients with 11q deletion, and by all 5 patients with neither deletion.

Immune modulation

Another potential treatment regimen involves the immunomodulatory agent lenalidomide, with one study reporting complete and partial responses (ORR 32%) after lenalidomide treatment in relapsed/refractory CLL.[15] Some researchers have suggested that a novel immune dysfunction exists in T cells from patients with CLL; repair of immune synapse defects may be an essential step in improving cancer immunotherapy approaches, they say.[16] In the presence of lenalidomide, synapse formation improved in the autologous T cells and CLL cells. Future clinical trial data may shed some light as to how lenalidomide works.

R2: Revlimid® plus rituximab

Chemotherapy-free treatment is under investigation. Unpublished data from a cohort of previously untreated patients at MD Anderson Hospital demonstrate a 90% CR rate in low-grade lymphoma after treatment with Revlimid (lenalidomide) plus rituximab. This approach is currently being trialled in previously untreated CLL: a preliminary ASCO report on 37 patients showed tumour flare in 21 patients. No patient had to be removed for progressive disease. Other effects included fatigue, transaminitis and myelosuppression.

Cell therapy in CLL may prove beneficial: cells can be used for direct anti-leukaemic activity and for anti-infection activity. A phase 2 trial that investigated the long-term outcome of reduced-intensity conditioning allogeneic stem cell transplantation (alloSCT) in patients with poor-risk CLL reported long-term MRD-negative survival in 27 (52%) of the 52 patients with MRD monitoring available, independent of the underlying genomic risk profile.[17] Conditioning was fludarabine/cyclophosphamide-based. After a median follow-up of 46 months, 4-year nonrelapse mortality, event-free survival (EFS) and OS for all 90 patients who received alloSCT were 23%, 42%, and 65%, respectively.

Personalised therapy with T cells

Cell types include cells capable of recognising minor histocompatibility antigens, and cells recognising tumour-specific or tumour-associated antigens, with T cells expressing chimeric antigen receptors for CD19. Over the last few years, clinical trials have begun to use chimeric antigen receptors (CARs), anticancer entities consisting of a single-chain antibody fragment, specific to a tumour-associated antigen, fused to a component of the T cell receptor complex (typically CD3zeta) that on antigen binding primes the engrafted T cell for anti-tumour activity (as shown in Figure 1).

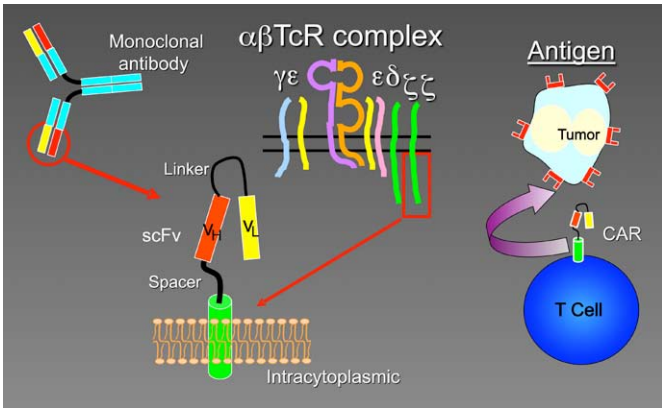

Figure 1. Chimeric Antigen Receptor (CAR) structure

To date, studies with first-generation CD19, CD20 and GD2 CARs have shown a lack of persistence of transduced T cells once infused.[18] This may be due to the fact that tumours lack ligands for co-stimulatory molecules and provide incomplete activation signals to the CAR-expressing T cell. Intracellular domains of co-stimulatory molecules have been introduced into the modular structure of CARs, in an attempt to recapitulate physiological activation of T cells to produce second- and third-generation CARs (see Figure 2 next page). Secondary endodomains include CD28, CD134 (OX40), CD127 (IL-7R), ICOS, DAP10 (NKC activation). Enhancing activation, proliferation and persistence of T cells in this manner may, however, potentially produce a supraphysiological stimulus leading to activation-induced cell death of the T cell and potentially harm the patient. Virus-specific cytotoxic T lymphocytes (CTLs) and CARs have been introduced, intended to lead to CTL activation and expansion.

However, evidence from US-based clinical trials infusing CAR+ T cells have revealed therapeutic limitations, including lack of persistence, homing, inadequate activation or tumour suppression of activity. Potential solutions for overcoming these limitations include modifying T cells to enhance persistence and homing, enhancing tumour antigen expression, and modifying the CAR to enhance activation.

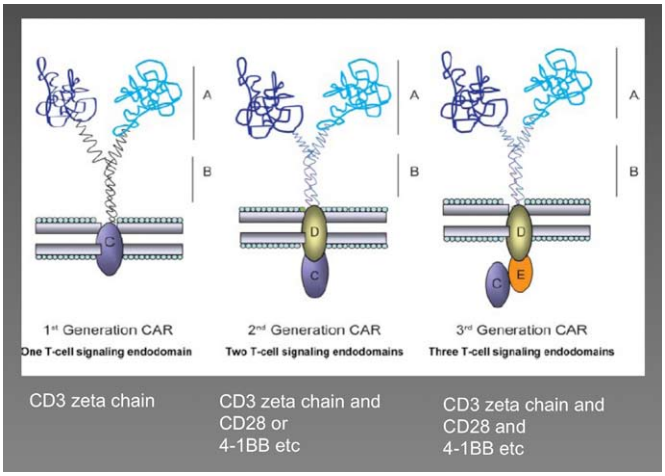

Figure 2. CAR Design and Evolution. Figure from Jena et al., Blood 2010; 116(7):1035-44.[18]

Concluding remarks

- For patients with MRD-negative CR or MRD low-positive CR, maintenance strategies using monoclonal antibodies/IMiDs look likely to be of benefit
- For patients with SD/PD on best available (purine analogue-based) therapy, addition of novel agents will be required e.g. alemtuzumab/ABT-263/flavopiridol/high-dose chemotherapy, but post-induction maintenance will be necessary and cell therapy may assist in dealing with infectious complications
- For older patients (and in the future maybe all patients) chemotherapy-free regimens may be best: R2 looks promising
- CD19 CAR-bearing T (or other) cells are showing early promise: their role in therapy is undefined and could be upfront or in MRD maintenance.

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