Simplified one-step antibody-HLA directed expansion of HIV-specific cytotoxic T lymphocytes: a system suited for use in vivo

The activation and expansion of specific anti-HIV CD8 cytotoxic T lymphocytes (CTL) that recognize clinically important HLA class I/peptide complexes has significant potential clinical applications. However, approaches that generate specific CTL generally rely on the production of large quantities outside the patient for subsequent adoptive transfer, a process that is labour intensive, expensive, cumbersome and susceptible to contamination [1–5].

With the aim of producing CTL expansion in vivo, we have recently described the use of a two-step antibody delivery system for the delivery of pre-formed HLA/peptide complexes to the abundant CD20 molecules on the surface of B cells, co-opting the role of B cells as antigen-presenting cells [6]. Using this sequential system in patients’ unseparated peripheral blood mononuclear cells (PBMC) ex vivo, significant expansion of anti–Kaposi’s sarcoma-associated herpesvirus (KSHV) and anti-HIV-1 tetramer-positive CD8 positive lymphocytes was observed. It has previously been demonstrated that CTL produced by this method have lytic ability [7], and are able to secrete IFN-γ as detected in enzyme-linked immunospot assays.

We are planning to test the safety and therapeutic ability of this system in HIV-1-infected individuals. However, to avoid the need for sequential injections and to simplify the process to a one-step procedure, the anti–CD20 B9E9 scFvSA fusion protein could be pre-bound to biotinylated recombinant HLA-A2 class I monomers containing the HLA-A*201 restricted Gag peptide (SLYNTVATL) (Fig. 1) ex vivo, and then administered as a preformed complex. To explore this simplification we have tested the ability of this modified protein to expand HIV-1-specific CTL ex vivo in a one-step procedure.

To achieve this, the tetravalent B9E9 scFvSA fusion protein (Aletheon Corporation, Seattle, USA) was joined to the HLA-A2/Gag complexes in vitro by mixing at a molar ratio of 1:4, to produce tetravalent scFv-streptavidin HLA class I complexes. Analytical gel filtration chromatography demonstrated that the tetravalent scFv-streptavidin/MHC complexes were essentially homogenous, with less than 5% of total protein consisting of free monomeric MHC complex.

PBMC were incubated with the protein (10 µg/ml) for one hour at 4°C. The cells were then washed and placed into 24-well plates at 2.5 × 10⁶ PBMC/well and cultured in RPMI with 10% fetal calf serum, l-glutamine and penicillin/streptomycin. IL-7 (R&D Systems, Minneapolis, MN, USA) was added on day 1 at 10 ng/ml, and IL-2 (Chiron, Harefield, UK) was added at 10 U/ml on day 4. For further stimulation, fresh PBMC were obtained from the same patients at weekly intervals and treated as above. The new autologous ‘immunized’ cells were then mixed with the existing cells at a 1:2 ratio, and culturing continued for a further 7 days in 5% carbon dioxide at 37°C (Fig. 1a).

In consecutive HLA-A2-positive HIV-1-positive individuals, the single-step ex-vivo immunisation procedure resulted in clear expansion of tetramer staining CD8-positive T cells after a single stimulation cycle, a situation not observed previously (Fig. 1b). The tetramer-positive CD8-positive cells were CD27/28+/69+/45Ra⁺, and the percentage of gated tetramer-specific cells that fell into this category approached 100%, significantly greater than previously (Fig. 1c). HIV-negative HLA-A2-negative individuals failed to show such expansion.

These data strongly suggest that a one-step procedure may be optimal both in terms of expansion and patient preference. Previous theoretical concerns regarding the potential for stearic hindrance with this larger protein appear not to have been confirmed. This one-step system is able to link to B cells via the anti-CD20 scFvSA fusion protein, and subsequently induce the expansion of specific CTL taking advantage of the co-stimulatory molecules present on B cells. As we have previously shown that dendritic cells are depleted in HIV-1 [8] (and KSHV [9] infection), B cells appear to be particularly suited to antigen presentation in this setting. Here, we observed significant and sustained expansion of anti-HIV CTL within ex-vivo PBMC. Although we have shown results ex vivo, the methods we describe lend themselves to in-vivo work. As a minimum, such studies will help delineate the importance of an effective CD8 CTL response. In one notable case, a good CTL response against nine known HIV-1 epitopes did not protect an individual undergoing his third supervised treatment interruption from superinfection with a highly related HIV-1 sub-type [10]. As such, we do not know, however, whether an effective, specific anti-viral response is beneficial in the
majority of infected individuals, and whether the therapeutic vaccine proposed here will be able to modulate the course of disease.

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Fig. 1. Expansion of HIV-specific cytotoxic T lymphocytes using the one-step antibody-HLA system. (a) A cartoon demonstrating the one-step B-cell bound monomer-fusion protein procedure used. (b) Facs plots for a single patient demonstrating an increase in cytotoxic T lymphocytes to HIV after two cycles. Left, background; middle, cycle 1; right, cycle 2. (c) The tetramer-positive CD8-positive T cells were further phenotyped, and all were found to express CD45Ra and CD69 (right) with absent CD27 and CD28 (left).
We read carefully the recently published above review [1]. Despite its importance in designing strategy for the management of HIV–hepatitis C virus (HCV) co-infection, this review gives an incorrect account of some previous published studies.

Table 3 referred to the studies by Perronne et al. [2] and Cargnel et al. [3], which have been published as abstracts, were presented at two international conferences, and were included in the listed references as numbers 59 and 62.

The results of both studies reported some mistakes in the number of patients and in the percentages of those achieving a sustained virological response.

The first study in particular [2] reported preliminary data on the Ribavic trial, the definitive results of which were presented at the recent Conference on Retroviruses and Opportunistic Infections, 2004. The table reported that 100 treated patients had an overall sustained response rate of 38%, with a percentage of 25% for genotypes 1–4 and 42% for genotypes 2 and 3. However, the abstract presented at the XIVth International Barcelona Conference on AIDS reported that 110 patients were treated with pegylated interferon alpha 2b (peg-IFN-α2b), and the virological response at week 48, at the end of treatment and not a sustained response, was 44%, with 19% for genotypes 1–4 and 57% for genotypes 2 and 3. The definitive data referring to sustained response were presented at the Conference on Retroviruses and Opportunistic Infections, 2004 [4], and corresponded to 26% overall in the peg-IFN-α2b arm.

In the same way, the data reported in the study by Cargnel et al. [3] referred to results presented at the Digestive Disease Week, 2002, which was not published later. The abstract presented reported that the number of patients treated with peg-IFN-α2b was 20, of whom 10 achieved a virological response, but not specifying at what time, and the different subtype percentages of response were not reported in the abstract.

As the treatment of HIV/HCV co-infection is presently one of the major topics, we hope that this Journal will clarify these incongruities, which could have an impact on clinical choices.

We also suggest that the article should be updated, because at the latest Conference on Retroviruses and Opportunistic Infections the results of the largest trial so far, APRICOT [5], were presented. The results of that trial show that, using peg-IFN-α2a plus ribavirin, the overall rate of sustained virological response was 40%, with 29% for patients with HCV genotype 1 and 62% for those with genotypes 2 and 3. These results can dramatically change the clinical approach to the management of HIV–HCV co-infection, and need to be known by every clinician before beginning the treatment of patients with co-infection.

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Reply to Bruno et al.: update to HIV–hepatitis C virus consensus guidelines

We would like to thank Bruno and colleagues for their interest in the updated recommendations from the HIV–HCV International Panel published in the journal earlier this year [1]. The authors mentioned that there are some incorrect figures in two of the studies recorded in the table, in which the results of trials using pegylated interferon (peg-IFN) plus ribavirin are summarized. However, the two trials were ongoing at the time the manuscript was submitted for publication. Two of the main investigators of those trials were members of the panel and they updated their results at the time the manuscript was written, which explains the apparent discordances found by Bruno et al. looking at abstracts presented at older conferences. The final results of one of those studies, the French RIBAVIC trial, were recently presented at the 11th Conference on Retroviruses and Opportunistic Infections [2]. Moreover, more updated results from the other study were presented at the Digestive Disease Week, 2003, which took place in Orlando in May 2003. In that Italian trial, 69 patients were randomly assigned to receive peg-IFN plus ribavirin; the virological response at 24 weeks had been reached by 19 of the 34 co-infected patients (56%) treated until that moment [3].

The authors ended their comments highlighting the results of the APRICOT trial, another study presented at the latest Retrovirus Conference, in which peg-IFN plus ribavirin was used for treating hepatitis C virus (HCV) in HIV–co-infected patients [4]. Although the results from that trial are the best obtained so far, Bruno et al. should not ignore the fact that the response rates are much lower than in HCV-monoinfected patients. Moreover, other studies in co-infected individuals have provided even lower response rates using similar treatment regimens, including another two trials presented at the 11th Conference on Retroviruses and Opportunistic Infections (see Table 1). We would like to take the opportunity in this letter to update and discuss briefly the most recent data on this matter.

The ACTG 5071 included 66 co-infected patients from several US centres, who were treated with a fixed dose of 180 µg/week of peg-IFN alpha-2a (Pegasys) plus ribavirin [5]. All subjects began ribavirin at doses of 600 mg per day and increased up to 1000 mg at week 12 if the tolerance was acceptable. In this trial, 77% of patients carried HCV genotype 1, which tended to respond less well to HCV therapy. End-of treatment response was reached by 41% of patients, but sustained virological response was only maintained by 27% (14% in patients with HCV genotype 1 and 73% in patients with other genotypes).

The previously mentioned RIBAVIC trial was a multicentre French study conducted by the Agence Nationale de Recherche sur le SIDA, in which 205 co-infected patients were treated with a weight-adjusted dose (1.5 µg/kg per week) of peg-IFN alpha-2b (PegIntron) plus a fixed dose of 800 mg ribavirin [2]. Overall, 27% of patients reached a sustained virological response (15% for HCV genotypes 1 or 4, and 43% for genotypes 2 or 3).

The APRICOT is the largest trial conducted so far in co-infected patients assessing the response to current HCV therapy. A total of 289 patients received at least one dose of peg-IFN alpha-2a (Pegasys) 180 µg/week plus a fixed dose of 800 mg ribavirin per day [4]. In contrast with the previous two trials, that study was conducted by the industry (Roche) and included patients from several countries and continents. This was reflected in a lower proportion of patients with HCV genotype 1 (60%) in that trial with respect to the others. The overall rate of sustained virological response was 40%, but dropped to 29% among patients with HCV genotype 1. A close monitoring of patients and strict inclusion criteria provided a relatively low discontinuation rate in that trial (25%), whereas in the French RIBAVIC study up to 36% of patients did not complete therapy [2].

The reasons for the poorest response in HCV/HIV-co-
infected patients with respect to HCV-monoinfected individuals are many, and have already been discussed in more detail in the HIV–HCV consensus guidelines [1]. Clearly, new treatment schedules and hopefully new drugs should be investigated to improve the response rates in co-infected patients.

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### Table 1. Main features and results of the three trials presented at the 11th Conference on Retroviruses and Opportunistic Infections using pegylated interferon plus ribavirin for the treatment of hepatitis C in HIV-positive patients.

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<tr>
<td>No. of patients</td>
<td>66</td>
<td>289</td>
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<tr>
<td>Intravenous drug users</td>
<td>80%</td>
<td>62%</td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td>11%</td>
<td>15%b</td>
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<tr>
<td>HCV genotypes 1 or 4</td>
<td>77%</td>
<td>67%</td>
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<tr>
<td>Mean CD4 cell count (cells/µl)</td>
<td>492</td>
<td>520</td>
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<tr>
<td>Patients on antiretroviral therapy</td>
<td>85%</td>
<td>84%</td>
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<tr>
<td>Earlier discontinuations</td>
<td>12%</td>
<td>25%</td>
</tr>
<tr>
<td>End-of-treatment responsea</td>
<td>41%</td>
<td>49%</td>
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<tr>
<td>Sustained virological responsea</td>
<td>27%</td>
<td>40%</td>
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aResults based on intent-to-treat analyses.

bCirrhosis or bridging fibrosis

### References


### Human resources in scaling up HIV/AIDS programmes: just a killer assumption or in need of new paradigms?

In the countries hardest hit by HIV/AIDS, the pandemic’s onslaught on the health workforce institutes a vicious circle that puts the health services under ever greater pressure. Unfortunately, these services have little reserves left and chronic deficiencies regarding training, recruitment and retention in the medical professions, unequal distribution and poor skill mix strain their performance. With the international agencies now bankrolling the scaling up of antiretroviral therapy and the price of drugs dropping continuously, the health workforce stands to make or break any programme.
troviral therapy pilot project among other activities and employs two (expatriate) doctors, three clinical officers and 14 nurses, some of whom were recruited in neighbouring districts. At the end of 2003, 8 months after the start, 385 patients were on antiretroviral treatment in Thyolo district (A. Chantulo, personal communication). This represents an annual uptake capacity of approximately 600 new patients. In this setting, the ‘perfect’ programme, treating all AIDS patients, would have an annual uptake of approximately 7000 patients, accompanied by a corresponding increase in staff. However, this scenario does not even consider the need to provide preventative interventions, or to take care of the other unabated health problems such as malaria, tuberculosis and reproductive health, with which the health services have to deal on a permanent basis.

Two fundamental issues are therefore emerging. The short-term priority is to adapt the health service delivery and organization to make the best use of current resources, for example by considering the integration of antiretroviral care in existing tuberculosis directly observed therapy programmes [3]. Basically, new delivery models should allow for the delegation of tasks to lesser-qualified health workers and lay persons, supervised by the increasingly scarce professionals.

However, the long-term priority is to institute effective human resource policies to train and retain the required health workers. In Malawi, where the vacancy rate in public health services is estimated to be approximately 60%, simply topping up the public servants’ salaries and hiring some expatriate professionals will not do to fill in chronic and structural deficits.

The hard decisions governing the human resource policies in the health sector are taken not in the Ministry of Health, but in the Public Service Commission and the Ministry of Finance. Through their aid programmes, international agencies are also important actors. Poverty reduction strategy papers are a good indicator of the importance given to the health workforce by both governments and international actors. Disappointingly, a review of the poverty reduction strategy papers – heavily indebted poor countries initiative in six selected African countries showed that in the best case the human resource crisis is merely acknowledged, and that an in-depth analysis of the issue and how it relates to civil service conditions is conspicuously absent [4]. A recent World Bank review came to the same conclusions [5]. In south-eastern Africa, where entire societies are in a process of what De Waal called social involution of a scale probably unprecedented in human history [6], this silence is deafening. What we need are paradigm shifts and approaches that used to be politically correct in other times and now urgently need to be reconsidered.

On a macro level, the opportunities offered by poverty reduction strategy papers need to be maximally exploited and should include a human resource development plan that rallies all the stakeholders. Recruitment ceilings imposed by structural adjustment programmes and similar donor-imposed conditions need to be lifted, whereas the global initiatives such as the global fund should actively seek to contribute to the expansion and stabilization of the health workforce. Simultaneously, approaches to expatriate technical assistance that used to be ‘developmentally’ sound in past conditions now simply reduce the effectiveness of international aid. In high prevalence countries, the autonomous sustainability of programmes can no longer be a constraining condition. Importing health professionals from countries with excess capacity for clinical and managerial roles is a short-term priority, if the funding flow is not to exceed the absorption capacity. All the while, innovative service delivery models need to be developed, whereby the challenge is to think outside the box of the pilot setting.

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Addressing the educational void during the antiretroviral therapy rollout

There are 40 million HIV-infected individuals globally [1], the majority of whom live in developing countries where, until recently, antiretroviral therapy (ART) was not available. With the global push to improve access to drugs in the developing world, ART is becoming a reality. The current challenge becomes how to deliver ART effectively because misuse will have drastic consequences.

The Moi University Faculty of Health Sciences (MUFHS) is one of two medical schools in Kenya (population of 32 million; estimated HIV prevalence rate 15%) [2]. MUFHS has, since its inception, been partnered with US institutions (Indiana University School of Medicine and, later, Brown Medical School) to foster collaborative medical exchange programmes. The collaboration responded to the HIV epidemic with the formation of the Academic Model for the Prevention and Treatment of HIV/AIDS (AMPATH). Using the AMPATH blueprint, MUFHS opened its first HIV treatment clinic with philanthropic donations in December 2001, with a modest goal to treat 50 patients. From this beginning, additional programmes developed: three HIV clinics at affiliated rural health centers, a core laboratory (capable of measuring CD4 cell counts and viral loads), a prevention of mother-to-child transmission programme leading to a mother-to-child transmission-plus grant (Mailman School of Public Health, Columbia University). Currently, over 2000 patients receive care at AMPATH HIV clinics; 50% are on ART.

The MUFHS faculty recognized early that personnel represented a critical component of the infrastructure. However, few if any Kenyan healthcare providers had any background knowledge, experience, or training in HIV care. At the opening of the HIV clinic, Kenyan providers were mentored by US collaborators. US collaborators offered expertise in the use of ART; the Kenyan faculty provided expertise in local resource management. Together they developed protocols on the best use of ART and the treatment of opportunistic infections in this resource-poor setting. Knowledge was forged to ensure that scarce resources were not squandered and patients were not lost through missed opportunities. The current challenge is to disseminate their knowledge rapidly and effectively as demands for HIV care increases.

Experience at MUFHS HIV clinics demonstrated that one clinical officer with physician supervision could see 30 HIV patients per day, 150 visits per week, a total of 7000 visits per year. MUFHS serves a catchment area of 13 million individuals (Fig. 1). Applying a local HIV prevalence of 15%, 980 000 HIV-infected individuals currently require care. Conservatively, 15% of these patients have a CD4 cell count less than 200 (CD4% < 15) or an opportunistic infection. These patients require at least 12 visits per year, either to monitor ART adherence/side-effects or to monitor and treat for opportunistic infections in patients without ART access. The 85% of HIV infected patients with CD4 cell counts greater than 200 (CD4% > 15) will require four visits per year to monitor general health and immune function. A total of 5.2 million visits per year will be needed to provide this routine care. Using these estimates, MUFHS today requires 730 clinicians trained in HIV clinical care to serve their catchment area. This calculation is not inclusive of the nurses, pharmacists, nutritionists and outreach workers required to provide appropriate multidisciplinary HIV care.

In response to this educational void, the AMPATH: HIV Clinical Care Training Programme was created. This programme consists of two modules: a didactic training programme covering issues such as HIV pathogenesis, diagnosis, laboratory monitoring, the pharmacology of ART, the prevention/treatment of opportunistic infections, tuberculosis, pediatric HIV

![Fig. 1. Projection for HIV care visits in western Kenya.](image-url)
care, and psychosocial wellbeing. Part two is a mentored internship in the HIV clinics of the AMPATH programme.

The first training programme, held in September 2003, trained 30 healthcare workers (physicians, clinical officers, nurses, nutritionists, outreach workers and pharmacists). Subsequently, four additional courses have occurred, with plans to scale up the training programmes at the request of the Kenyan National AIDS and STI Control Programme.

This programme is described as a model. A critical, early component of ART programmes must be the training of healthcare professionals to supply quality care in resource-limited settings. Without this, the expected outcome will be inappropriate drug prescribing patterns, the inadequate evaluation of adherence, missed opportunities to prevent opportunistic infections, and missed opportunities for HIV prevention. Poor education of service delivery personnel will directly result in the development of drug resistance, the loss of patient lives, and ongoing transmission. The delivery of ART drugs cannot occur in an educational void.

The faculty of MUFHS urges that an educational training assessment be an early priority in areas in which ART delivery is occurring. The consequences of ignoring this educational void may be too devastating to overcome.

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