

# COMMENTARY

## Long-term findings of HIVNET 012: the next steps

See page 859

In 1999, the HIVNET 012 team reported exciting preliminary results that single-dose nevirapine prophylaxis for mother and baby significantly lowered HIV-1 infection risk at 14–16 weeks compared with controls who received short-course zidovudine prophylaxis.<sup>1</sup> The report in today's *Lancet* by the HIVNET 012 researchers shows that this benefit persisted to 18 months of age. Toddlers from the nevirapine arm had significantly lower risk of HIV-1 infection (15.7% vs 25.8%) and higher probability of HIV-1-free survival (80% vs 70%).

Since 1999, much has been learned about transmission prophylaxis and treatment of HIV-1. Transmission prophylaxis that is effective in one setting<sup>2</sup> may not be effective in another, and successful prevention of early transmission may not result in decreased risk of HIV-1 infection later in childhood.<sup>3</sup> Detectable resistance to nevirapine will emerge in 19% of women 6–8 weeks after receiving a single 200 mg nevirapine pill, and in 46% of nevirapine-exposed infected infants.<sup>4</sup> Investigators from Zimbabwe<sup>5</sup> have detected 75% nevirapine resistance in samples drawn from women within 2 weeks after single-dose nevirapine prophylaxis. Given the technical challenges of detecting resistance mutations in minority populations and mutations archived in latent T-cell reservoirs, the actual number could approach 100%.

The HIVNET 012 protocol did not affect child survival. Overall infant mortality at 18 months was 12% and did not differ significantly between groups (10.6% with nevirapine and 13.6% with zidovudine,  $p=0.253$ ). Other investigators in sub-Saharan Africa have also noted high mortality in the infants of HIV-infected mothers<sup>6,7</sup> and have proposed two causes: perinatal HIV-1 exposure and debility of the caregiver.<sup>8</sup> We have ample data proving that nevirapine prophylaxis can prevent vertical transmission when women have no access to other antiretrovirals, but understanding of debility of the caregiver is extremely limited.

New studies oriented toward care of entire families living with HIV-1 are in progress.<sup>9</sup> They will attempt to reduce both vertical transmission rates and debility of the caregiver by treating parents (including pregnant mothers) with potent combination therapies. In the meantime, what can be done to improve the survival of infants of infected mothers if transmission prevention alone has no effect on 18-month mortality?

First, we must do no harm. The HIVNET 012 researchers predict that universal implementation of single-dose maternal and baby nevirapine prophylaxis could prevent 246 000 of 800 000 paediatric HIV-1 infections a year. It would leave between 20% and 100% of the 3.2 million mothers who received prophylaxis and more than 50% of the remaining 554 000 infants infected (despite prophylaxis) and resistant to nevirapine and other non-nucleoside reverse-transcriptase inhibitors—the very agents on which success of antiretroviral therapy in resource-poor settings will hinge. HIV caregivers know well how to avoid

induction of resistance to non-nucleoside reverse-transcriptase inhibitors. When these potent and highly effective agents are used in combination with other agents (usually two nucleoside reverse-transcriptase inhibitors), resistance is unlikely to emerge. When non-nucleoside reverse-transcriptase inhibitors are used alone, rapid emergence of resistance is almost certain,<sup>10</sup> even when used for one dose.<sup>5</sup> Why not modify the HIVNET 012 protocol to forestall emergence of resistance? For example, 2–3 days of zidovudine and lamivudine could be added to the single maternal nevirapine dose. Generic starter packs are already available for combination therapies. Design and manufacture of small perinatal prophylaxis packs would be straightforward. A study of such a strategy is in development in the Pediatric AIDS Clinical Trial Group in the USA, but by the time those results are available, more than 10 million women living in resource-poor settings could already have been rendered nevirapine-resistant by vertical transmission-prophylaxis protocols.

Second, we must exploit available data sets and cohorts to gain more insight into correlates of morbidity and mortality of HIV-1 exposed children. HIVNET 012 apparently did not, and will not, collect data on mothers beyond 8 weeks post-partum. Three women died within 56 days of delivery—all with clinical AIDS. How did their infants fare? When might interventions with combination antiretroviral therapies or treatment of opportunistic infections have made a difference for these mother-baby pairs? HIVNET 012 mothers will remain important study participants as they bring their children back for follow-up. Simple observations, such as determining who brings children to follow-up visits would reflect death and disability of participating mothers—potentially important co-variables in the welfare of study children.

Third, we must all abandon the notion that universally acknowledged principles of antiretroviral therapy do not apply to HIV-1-infected pregnant women. The HIVNET 012 researchers and others have suggested that resistance to potent antiretrovirals used alone<sup>1</sup> or in suboptimum combinations<sup>11</sup> for vertical transmission prophylaxis may “fade” and have no effect on future treatment options. Such a hypothesis needs examination; however, all evidence to date shows that once resistance to an agent is detected, it will remain with an individual indefinitely.<sup>12</sup> In the case of non-nucleoside reverse-transcriptase inhibitors, induction of resistance is likely to render the entire class of drugs virtually useless as AIDS therapy.<sup>13</sup>

Today's report contains a reiteration of a US Public Health Service recommendation<sup>14</sup> that nevirapine single-dose prophylaxis be used for vertical transmission prophylaxis in women in more developed countries who are diagnosed with HIV-1 infection close to or during labour, perhaps in combination with zidovudine prophylaxis. It is disturbing that no man would be intentionally exposed by his caregivers to nevirapine or zidovudine, either alone or in a dual combination. Why

should this ever be an acceptable strategy for any infected individual?

Finally, we can no longer delay the adoption of an international standard of care for all individuals living with HIV-1, regardless of their reproductive status and where they live. Marginalised populations, including the homeless and the displaced urban and rural poor, are quite capable of adhering to effective HIV therapies. Adherence rates in several African settings surpass those of the developed world.<sup>15</sup> Last week the world witnessed the official opening (sanctioned by the World Trade Organisation) of the first fissure in international trade barriers to the global distribution of inexpensive generic antituberculosis, anti-malaria, and antiretroviral agents.<sup>16</sup> Generic antiretrovirals, prepared in convenient single-pill triple combinations for once and twice daily dosing, are now available for less than US\$1 a day. The HIVNET 012 protocol was modified in 1998 to accommodate the changing realities of the HIV-1 pandemic. 5 years later, these realities have shifted yet again. Suboptimum single-agent and double-agent prophylaxis protocols no longer have a justifiable place in the front lines of the global struggle against HIV/AIDS. It is up to all of us to focus on development of equitable distribution and effective use of these agents now. Once they are widely available, it may be too late.

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## Membrane-repair machinery and muscular dystrophy

Disruption of the plasma membrane is a common event in various normal animal cells, and membrane-repair machinery is essential to prevent disruption-induced cell death. In skeletal muscle, membrane disruptions are most often observed under physiological conditions, because muscle fibres contract repeatedly and are often susceptible to varying degrees of mechanical stress. The fragility of the sarcolemma makes it unable to withstand the mechanical stress, and a defective membrane-repair easily results in necrosis of the muscle fibres.

Muscular dystrophy is characterised by progressive muscle weakness and wasting, with necrosis and regeneration of muscle fibres. Since dystrophin was identified as the gene product responsible for Duchenne muscular dystrophy (the most common form of muscular dystrophy) in 1987, several muscular dystrophies have been associated with instability of the sarcolemma.<sup>1</sup> Lack of a component of the dystrophin-glycoprotein complex (DGC) at the sarcolemma results in instability of the entire complex and thus disrupts the structural linkage between the subsarcolemmal cytoskeleton and the extracellular matrix. Dysferlin is a sarcolemmal protein, and its deficiency causes proximal and distal forms of recessively inherited muscular dystrophies, designated as limb-girdle muscular dystrophy type 2B, Miyoshi's myopathy, and anterior compartment myopathy. Because dysferlin is a homologue of a nematode gene that mediates vesicle fusion to the plasma membrane in spermatids, it has been suggested that dysferlin is involved in membrane fusion.

Recently, Dimple Bansal and colleagues<sup>2</sup> investigated dysfunction in the membrane-repair machinery in muscular dystrophy. They produced dysferlin-null mice that had muscular dystrophy with a structurally stable sarcolemma and no change in the expression of DGC components. They showed that dysferlin accumulated at sites of membrane disruption in normal muscle (figure), although other sarcolemmal proteins, including caveolin-3 and  $\delta$ -sarcoglycan, were lost at the site of injury. When they directly assessed resealing efficiency, laser-damaged membrane of wild-type mouse muscle resealed within a minute in the presence of  $Ca^{2+}$ ; this  $Ca^{2+}$ -dependent resealing of injured sarcolemma was defective in dysferlin-null muscle fibres. These results suggest a direct role of dysferlin in the  $Ca^{2+}$ -dependent membrane-repair process.

Non-necrotic muscle fibres from dysferlin-deficient patients early on show membrane abnormalities, including small defects in the plasma membrane, replacement of the plasma membrane by one to multiple layers of small vesicles, and small subsarcolemmal vacuoles.<sup>3</sup> These findings are similar to those observed by Bansal and colleagues in dysferlin-null mouse muscle, which have accumulations of vesicles beneath the disrupted cell membrane. For rapid repair of the disrupted membrane, vesicles must accumulate and fuse with the plasma membrane. Abnormal accumulation of subsarcolemmal vesicles in dysferlin-deficient muscles indicates a defective membrane-fusion process.