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Subject: Cytometry and HIV in the Third World
E-mail circular on 30th October 2000

Ladies and Gentlemen-

This mailing seeks support - primarily moral, intellectual, and technical, but financial would be welcome, too - for a cooperative effort by the cytometry community and industry worldwide to aid in the struggle against the HIV epidemic in the Third World.

As those of you who have been involved in cytometry for many years know, it was the emergence of AIDS in the U. S. and Europe in the early 1980's that led to the widespread clinical use of fluorescence flow cytometry and the improvement of the previously shaky economic status of most of the instrument manufacturers. To borrow a phrase from the computer industry, HIV was, indeed, our "killer application". To their credit, many instrument manufacturers have attempted to produce smaller, more rugged, less expensive cytometric apparatus for clinical use in the Third World, but this has remained largely inaccessible to most of the African and Asian countries most affected by the epidemic.

I have spent several decades developing small, simple flow cytometers, and was recently asked by some investigators in need of an instrument for use of field trials of HIV vaccines in Africa whether I could produce a few prototypes for them. The request came just before the Cytometry Development Workshop, an event held every October at the Asilomar Conference Grounds in Pacific Grove, California, and attended by technical personnel from academic institutions, national laboratories, and industry. The Workshop has always had a strong ecumenical spirit, with information exchanged freely among competing laboratories and companies, and I felt that a cooperative effort might accomplish far more toward bringing affordable technology to regions in which it was desperately needed than I could by cobbling together a few more instruments. The following summarizes a discussion on the subject held at Asilomar; I have edited notes taken by Bob Murphy, who was co-chairman:

"The workshop participants discussed the need for low cost cytometers in Third World countries, especially in Africa. These are needed particularly for AIDS monitoring, especially in conjunction with HIV vaccine trials. The discussion initially focused on identifying preliminary design requirements. Since the primary need is expected to be for enumerating T helper (CD4+) cells in whole blood, a primary requirement is the ability to provide counts from a known volume (i.e., number of cells of a particular type per ml of blood). For flow systems, another primary requirement is that the system be capable of handling cells in whole blood. It may also be feasible to build an inexpensive image cytometer that provides volumetric counts, although such systems might require some type of pre-enrichment for white cells in order to get statistically meaningful counts for lymphocytes.

The participants then discussed the possible development of inexpensive cytometers (both flow and image) taking advantage of recent advances in relevant technology. The possibility of producing a system without a microscope objective to measure fluorescence (e.g., using fiber-optics or microfabricated optics) was discussed and ruled out due to the high background obtained when fluorescence is not collected with focusing optics (using fiber optics without an objective lens, light is collected from a region on the order of 1,000 times larger than with a typical objective lens). The possibility of devoting engineering effort to improving cell preparation methods (e.g., using robots) rather than reducing cytometer cost was discussed.

Systems currently under development were then discussed. Both the Guava system being developed by Guava Technologies and the Cell Tracks system being developed by Immunicon were considered to have good potential. The microfluidic systems being developed in Dr. Steven Quake's group at the California Institute of Technology are likely to reduce cost and complexity of flow cytometers in general, but current versions use rectangular fluid channels of 1-30 micrometers that would clog when using whole blood. Whether microsystems using fluid channels with dimensions of 100 micrometers or more can be made is uncertain.

The discussion then shifted to current commercial systems that might be suitable. The systems discussed included the FACScout manufactured by BD Biosciences (retail cost, \$35,000), the CyFlow manufactured by Partec (retail cost of appropriately configured instrument, \$25,000(?)), and the ZBI Coulter Counter manufactured by Beckman Coulter (retail cost, \$10,000). All are intended to be simple to use and can be run from a 12 volt battery. The first two are flow cytometers that use fluorescent-conjugated antibodies to identify cell types and, in the case of the FACScout, fluorescent beads to provide conversion to cell concentrations. The ZBI counts known volumes, and particular cell types can be identified by rosetting via specific antibodies. In all cases, there was concern that reagent costs would be high, especially for the fluorescence-based systems. The consensus of the group was that obtaining foundation or government support for purchasing existing instruments and reagents (at cost) might be more economical in the long run than the cost of efforts to design, manufacture and support a cytometer explicitly to meet the Third World need. Nonetheless, there was considerable enthusiasm that an inexpensive system could be designed and built with collaboration between cytometry experts inside and outside of industry, and there was a good deal of willingness among the participants to assist in such efforts."

There are two relatively long alphabetized lists of addressees on this e-mail. The first list includes the attendees of the Asilomar workshop; the second, culled from my files, from address lists of things other people have sent me, from business cards I have managed to unearth, etc., probably includes more of you who are wondering why you got this. I'll take the blame; it was because I feel you:

- 1) have influence in the cytometry community, industry, or both, and/or have a pipeline to people in your institution or company or elsewhere who have more and/or who have technical expertise, and/or
- 2) are involved in the development of cytometric apparatus or key components and could potentially contribute to the technical effort.

If you would prefer not to receive any further e-mail from me on this subject, just let me know. The rest of you can read on.

The summary of the discussion at Asilomar (forgive us for inaccuracies and misrepresentations) mentions several systems which might, either as designed or in slightly modified form, be suitable for the projected purpose and inexpensive enough to be sold in the Third World at a profit by their manufacturers, in which case there might be no need for the cooperative effort I have proposed. However, I suspect that the public health budgets of most affected countries are still inadequate to permit the purchase of adequate numbers of instruments.

Funds to combat the HIV epidemic and other public health problems of developing countries come from a number of sources, including the United Nations, World Bank, pharmaceutical companies involved in drug and vaccine

trials, and foundations, notably the Gates foundation. It might be argued that Bill Gates, if he had a mind to, could put multilaser cell sorters all over Africa, but the lack of power and cooling water would make it unlikely that many of them would ever be uncrated. The instruments that are needed should be designed for small size, ruggedness, and low power consumption, as well as minimal cost, and I suspect that there are limits to how far commercial organizations have taken this design concept, because it would be difficult for most of them to make a profit selling relatively small numbers of inexpensive machines.

A cooperative effort could produce a design that would be sold near cost for uses subsidized by the public and private funding sources mentioned; there would, however, be a potential commercial spinoff in that the inexpensive components and apparatus developed in the cooperative effort could be used in cytometric apparatus to meet the needs of price-sensitive emerging markets in developed countries, e.g., food and water microbiology. In terms of meeting the primary goal of getting affordable technology where it is needed in the Third World, the cooperative effort would indicate to potential funding sources that the industry and its technical partners were doing the right thing, increasing the likelihood of attracting financial support.

Where do we go from here?

If you'd like to join in, let me know; if the decisions about whether your organization can do this or not are above your level, pass this message up the line. If you're not interested, let me know and I won't bother you anymore.

If you think your company is shipping exactly the right stuff now, let me know, but I suspect that before the right stuff gets designed, we need to know what is needed in the field. Some of you on this list have the direct experience or the clinical contacts, and we need to bring together (first by e-mail, then conference calls, then face-to-face) those of us who know the clinical needs and those who can contemplate what technology is best suited to meet them. Eventually, we should look at clinical needs beyond the HIV epidemic; malaria comes to mind, but the emphasis will have to remain on supporting clinical research which will result in inexpensive therapeutic and preventive measures, such as the HIV vaccine trials which got all of this started.

Any technology that gets discussed should be something that can be shared without the necessity for nondisclosure agreements; it may very well be that an eminently usable instrument can be put together from subsystems that have been superseded in manufacturers' current products.

If this project gets off the ground, we ought to make it a memorial to Janis Giorgi, whose work in cytometry contributed a great deal to our understanding of HIV infection. Janis was always prodding those of us who developed new technology to get it into the clinic in a useful form as rapidly as possible, and I think she'd approve. I will make mention of this at a memorial symposium to be held in conjunction with the Clinical Cytometry Society meeting in Austin, Texas, November 12; if you will be in Austin and want to discuss the project, track me down (lab: 617-783-8392; house: 617-965-6044; cell phone: 617-283-6092).

Thanks,

-Howard

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