

### Dear Friends,

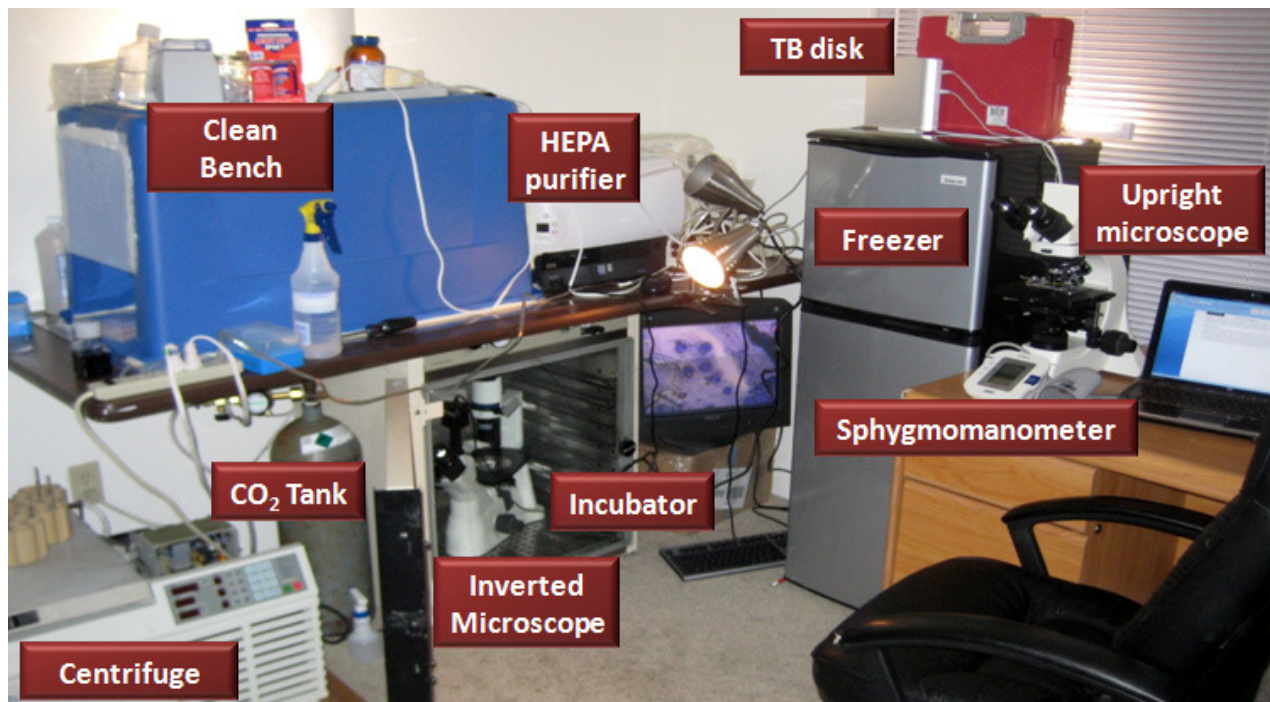
Welcome to the May issue of Livly's newsletter. Livly is a young non-profit we formed around the idea of using cells of the innate immune system to combat all sorts of cancer. Livly's work will draw largely on the work by Zheng Cui, who made headlines with the cancer-proof mice he discovered. However, Livly's work will be translational in nature – we will aim to fill key needs preventing this technology from being developed into a universal human cancer therapy.

While May brought challenges and a few disappointments, we cleared a major hurdle, namely building out our cell culture laboratory. The details of our challenges, successes and research results are below, sectioned into "Corporate and Science."

#### **Corporate**

In April, we applied for grants with the Edson Foundation and the Immortality Institute. While small, these grants would have allowed us to build a basic cell culture laboratory at Livly. In early May, we withdrew the smaller application to the Immortality Institute – there was a possible conflict of interest with the SENS Foundation, for which we both volunteer. Soon after, we received news that the Edson Foundation would not provide us with a grant. We were disappointed, yes, but far from defeated. We continued to plan our approach toward curing cancer, just without immediate grant funding. Then.... something amazing happened! John got very lucky trading options (see the company Dendreon), allowing him to buy the necessary equipment to set up the culture lab. (Figure 1).

**Figure 1: Livly's cell culture lab.** This is what a basic homemade cell culture lab looks like. All in all, this went for somewhere in the high four figures on eBay.

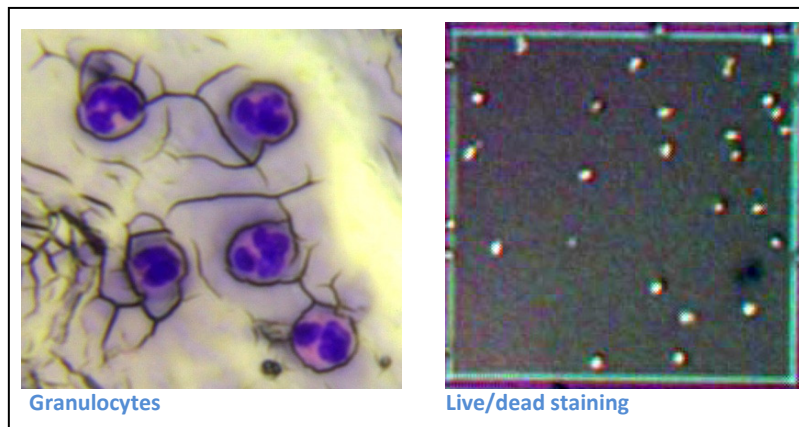


**Science**

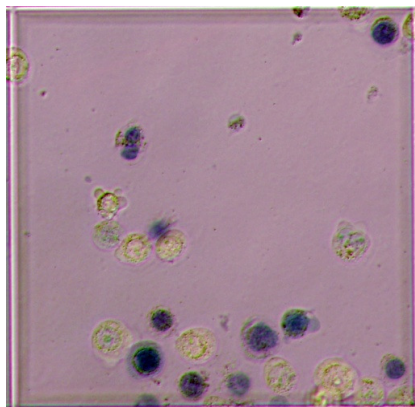
Granulocytes are the cells though to be responsible for cancer-immunity in a recently discovered cancer-proof mouse (See papers by Dr. Zheng Cui on PubMed). These mindless killer cells are loaded with an arsenal of destructive chemicals and enzymes, ready to take on any one of their pre-programmed targets. Seminal research by Dr. Cui showed that neutrophils from different people differ in their ability to recognize cancer cells. Ultimately, the goal of Livly's research is to identify people with exceptional neutrophil cancer-killing ability, and harness their granulocytes for a universal cancer therapy.

Using our new cell culture lab, we purified and characterized granulocytes from our own blood, for use in cancer-killing experiments. We found that we can indeed purify high quality granulocyte populations (Figure 2). These pure cells should be ready for use in cancer killing experiments.

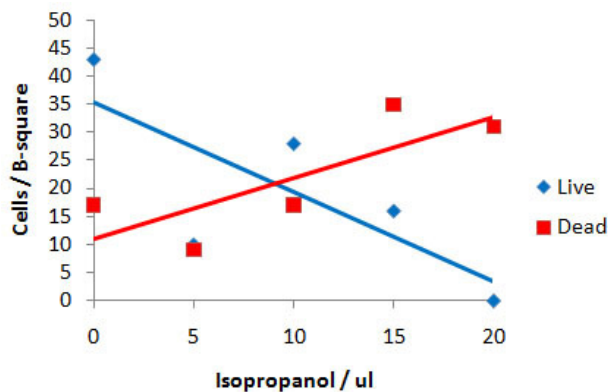
**Figure 2: Purified Granulocytes.** Our staining shows the characteristic fragmented cell nuclei (blue, left), confirming that these are indeed granulocytes. Furthermore, the granulocytes we purified are to a large extent alive (trypan blue staining, right). These images show that we can now purify intact human granulocytes from blood, a testimony to the functionality of our new cell culture lab.



In parallel, we began work to measure the killing of cancer cells quantitatively (Figure 3, 4). The cancer killing too is based on trypan blue, a simple dye that stains dead, but not living cells. This allows us to count how many cancer cells get killed by any agent in vitro. Initially, we used alcohol killing to validate the assay (Figure 4). Next, we need to do this with the purified granulocytes from Figure 2.



**Figure 3: Live-dead staining using trypan-blue.** Live cancer cells are white, dead cancer cells are blue. Easy, eh?



**Figure 4: Cancer-killing assay.** We killed cells with increasing dosages of alcohol, to test our killing assay. In this test run, we are getting some unpleasantly high variance. But we do pick out a trend – the more alcohol, the more dead cells, and the less live cells we see.



### Outlook

Our grant applications failed, but we built a basic cell culture lab anyway. This will keep Livly in business in the short to medium term. In the long-term, it is critical that we leverage this asset to access future donations or grants. Our best chance to do this is to focus on the following two simple sub-goals:

- Create a quantitative test to determine different people's neutrophil cancer killing ability.
- Capture persuasive images and time-lapse videos documenting cancer killing by neutrophils.

We have most resources we need to try these two things in the next couple of months. After that, we will need significant support.

Thank you for joining us in this challenge. We absolutely want to hear from you. Questions are encouraged!

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Livly, President and Co-Founder

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