|  |  |  |
| --- | --- | --- |
|

|  |  |
| --- | --- |
|

|  |
| --- |
| [What Method Can Be Used to Create a Cancer Cell and PBMC Coculture](http://www.conversantbio.com/e1t/c/%2AW1zq2g04whZ-xW3D3v_H2lHBDr0/%2AW8GZHh-4Wz2kYW4twLrn4N_dBc0/5/f18dQhb0S65P6XgRRNN12h5Nckl64FVDHvM05lZ5f1W8tCfJw5dDDLYW7RQBWN3M9d_DW4lH7dF9091l7W5dFz8b4YVvtyW5hdTXm3YwyGhW313NZd51MQjyW2h-XK48s0wx7W7NZwCZ3vlWy5W3337XB3SNs_1W6CbL4c7nYCsTW1rwNJr23Q-L6W1NSFtt5HWQF5N4HQ5z3z9x7BW4vqkWj1WnMYzW1hX-BF4-pZ6PMCqK9-449P2W4SF1h-4YwmvMW50l7B_5z-ZQrW1hhDRz6Q9yDTN6ttS9lVpynwN6cq40bmVb_KW6Fm1QM7mFQzJN71VyVnpXnTfW4S3vSr7pM2-zW2kzCXp6w_0MYW6yZNxn1FB4vhVYdRyz3g_nfLW2qx14d5xxB4JW2CTrNH2bP2SyW8H1TGy72yvfjW7-2fDm8VS0yhW4mC4-Q29LvGXW5pJ2mK51-sCqVVW1kZ7NmS0SW53jwn78-HNRnW8-jFCm7XM_qQW7pF4W53Dw77sN7pLNnH82ZTgW7818XD2CdlTKW2rDVHh1xSXfGW6Y6SYL2Xd8XNW2kCgb96bDyLXW4-39_17BZprNW6qmRLy6tvCGYVlX1Sr7C6W7K111)?*Posted by*[*Luke Doiron*](http://www.conversantbio.com/blog/author/luke-doiron)*on Aug 27, 2015 10:46:00 AM* |

 |

 |



Recent findings from the cancer research community have identified the importance of cell-cell communications in the activation or inhibition of tumor development. Cellular co-cultures have become an important in-vitromethodology for gaining a better understanding of how cells interact in the cancer microenvironment. These insights are also fueling development of drug modulators that effectively target the cell-to-cell dialogue that may fuel [cancer development](http://www.conversantbio.com/disease-areas/oncology-research-types-of-cancer) or therapy resistance.

A number of promising studies have utilized cancer and PBMC cell co-cultures, including these:

HER2 downregulation, immune effector cells and the mechanisms of action of trastuzumab

Trastuzumab is a monoclonal antibody therapy that targets HER2 overexpression of cancer, particularly in breast cancer. While this therapy has been used for over a decade, there is still a lack of understanding about its mechanism of action, especially in regards to the reasons behind the widespread resistance to this therapy. There have been many theories put forward, and [this recent study](http://www.breast-cancer-research.com/content/16/2/R33) aimed to provide greater clarification on this point.

Researchers used two co-culture techniques. In one, cancer cells from established cell lines were pre-seeded in a 24-well plate overnight in a RPMI1640 media, followed by the addition of PBMCs. These were added to the cancer cells at a ratio of 10:1 PBMC:Cancer cells and cultured at 37°C, then cells were separated for further analysis. In the second co-culture method, a transwell plate was used to keep cells in communication, but physically separated. Cancer cells were cultured in the lower chamber with or without trastuzumab and PBMCs were then added to the upper chamber. After 48 hours of co-culturing, PBMCs were removed from the top chamber and cancer cells prepared for western blotting analysis. Authors conclude that their study implies that HER2 downregulation in cancer cells treated by trastuzumab might predict "active engagement of [immune effector cells](http://www.conversantbio.com/life-science-researcher-resources/bid/360246/Researchers-Guide-to-Immunology) in the tumor microenvironment," which may help lead to better therapeutic use of trastuzumab.

Co-cultures to explore hepatocellular carcinoma expansion and suppression

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide, and treatments for many patients are limited. [This research](http://www.nature.com/labinvest/journal/v87/n6/full/3700540a.html) explored the role of CD4+, CD25+, and regulatory T cells (Treg) in HCC cancer and whether they positively correlate with tumor burden. Study method involved the use of PBMCs, from which CD4+ T cells were isolated. Next, negatively selected CD4+ cells were incubated with anti-CD25 microbeads and selected to obtain the CD4+, CD25+ and CD4+, CD25- fractions. Human hepatoma cell lines were cultured and the supernatants collected via centrifugation.

Co-culturing utilized transwell chambers; HCC cell lines and normal hepatocytes were plated underneath and incubated for 4 hours, followed by the addition of PBMC to the inner chamber. After 48 hours, PBMCs were harvested and stained for flow cytometry analysis or used to obtain CD4+CD25+ and CD4+CD25- cells.

To further evaluate upregulation of Tregs by HCC, authors prepared an in-vitro culture system using the Huh7 HCC cells, normal hepatocytes and PBMCs from healthy controls. PBMCs were separated using a transwell chamber.

Authors concluded that CD4+CD25+ T cells from PBMCs that were co-cultured with Huh7 HCC cells have a higher suppressor ability compared to those from normal PBMC cells, and that Huh7 culture supernatants seem to promote CD4+CD25+ T cell proliferation and inhibit CD4+CD25- T-cell proliferation.

General tips on co-culture methods

* Decide how you want to set up your in-vitro model. - For example, if you want to keep cells in communication, but physically separated, transwell plates are commonly utilized.
* When using transwell plates, pay attention to your membrane pore size, density and material.
* Optimize your growth surface for your primary tumor cells before attempting to co-culture.