

# Chang Marrow™: A fully supplemented, ready to use medium optimized for providing consistent growth for bone marrow cultures and the detection of abnormal malignancies

## Introduction

The advances in cancer genetics enable cytogenetics laboratories to use short-term cultures of bone marrow samples not only for the diagnosis of hematopoietic disorders but also for the prognosis of the specific tumors and the selection of effective treatments. Bone marrow aspirates are cultured for one, two or three days. During this short culture period, it is critical to obtain enough analyzable metaphase cells to complete cytogenetic studies. To achieve an optimum short-term cell growth, the culture medium needs to provide the right mixture of nutrients and growth factors. The commercially available bone marrow culture media contain a supplement of a cell conditioned medium as a source of hematopoietic growth factors as well as bovine serum. One commonly used supplement is Giant Cell Tumor conditioned medium (GCT- CM).

Below are two studies that compared the performance of a new, optimized bone marrow culture medium supplemented with GCT conditioned medium (Chang Marrow™) versus MarrowMax™, both intended for the growth of bone marrow cells and detection of abnormal cells by cytogenetic analysis.

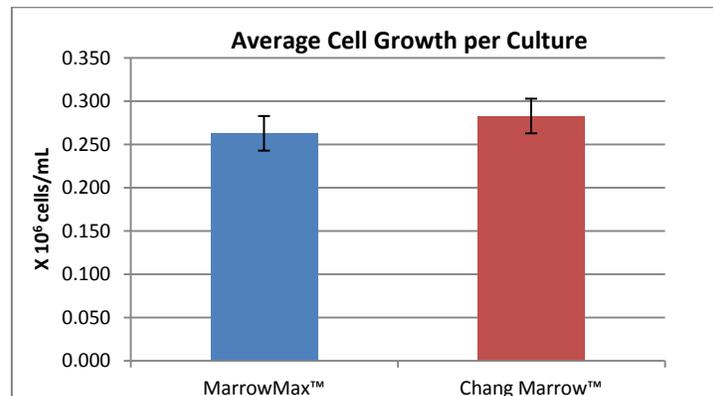
## Study One (TF-1 Assay)

TF-1 cell line (ATCC) was established from a heparinized bone marrow aspirate from a male with pancytopenia. The cell line is highly dependent on IL-3 and GM-CSF for growth and responds to other cytokines and lymphokines (IL-1, IL-4, IL-6, IL-9, IL-11, IL-13, SCF, LIF and NGF). Cell proliferation in test media was evaluated by culturing TF-1 cells (initial cell density inoculation  $3.0$  to  $5.0 \times 10^4$  cells /mL) in the test media and assessing the cell growth after 72 hours of culture.

### Results (Cell Proliferation):

The TF-1 cell line was used as a model for bone marrow aspirate to assess short-term cell division and growth. This cell line was chosen for its origin (hematopoietic lineage) and its dependence on and response to numerous cytokines and growth factors critical for in vitro bone marrow cell growth.

The performance of Chang Marrow™ was compared to MarrowMax™, results shown below (Figure 1).



**Figure 1:** TF-1 cell growth after 72 hours culture in Chang Marrow™ and MarrowMax™ (initial inoculation  $3-5 \times 10^4$  cells/mL). The results are the average of 5 separate experiments  $\pm$  SEM.

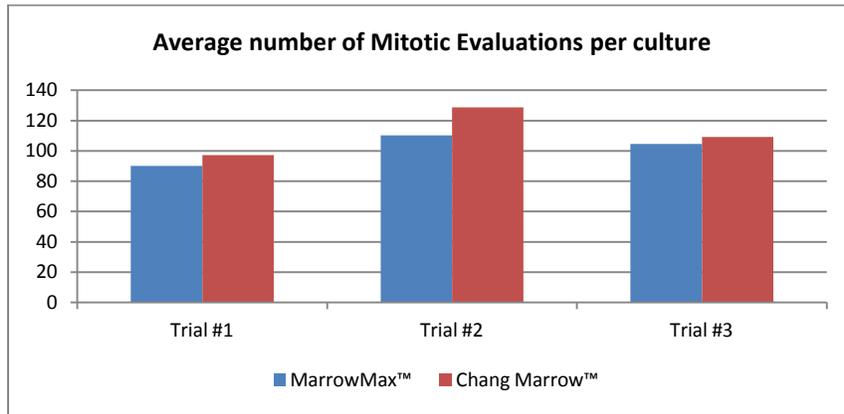
## Study Two (Clinical Evaluation)

The clinical evaluation of Chang Marrow™ was carried out by an independent clinical Laboratory (Colorado Genetics Laboratory) using their standard bone marrow culture protocol. Three lots were tested and for each lot of Chang Marrow™, a total of 20 patient samples were assessed. Each aspirate was cultured in parallel in both test medium (Chang Marrow™) and a control medium (MarrowMax™). The cultures were analyzed after

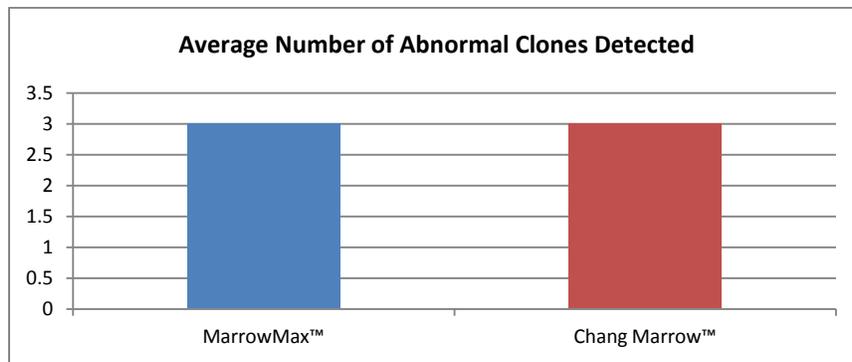
one, two and/ or three days of culture as needed to obtain at least 15 evaluable mitotic figures, and a minimum of 5 cells were analyzed for each sample. The following quantitative data were reported:

1. Number of mitotic figures (number of cells in mitosis that could be used for cytogenetic evaluation).
2. Average number of mitotic evaluations per culture.
3. Number of abnormal cells identified.

Results (Mitotic Index and Abnormal Cell Detection):



**Figure 2:** Results of clinical analysis of 3 different lots of Chang Marrow™. For each lot, bone marrow aspirate samples from 20 different patients were set up. The cultures were evaluated after overnight incubation, 2 days and/or 3 days as needed. The results reported here are the average numbers of analyzable mitotic cells per culture for each lot of Chang Marrow™ compared to MarrowMax™ on day 2 of culture.



**Figure 3:** Results of clinical analysis of 3 different lots of Chang Marrow™. For each lot, bone marrow aspirate samples from 20 different patients were set up. The cultures were evaluated after overnight incubation, 2 days and/or 3 days as needed. The results reported here are the average numbers of abnormal clones detected per culture for all 3 lots of Chang Marrow™ compared to MarrowMax™.

**Conclusion**

Chang Marrow™ is a new, high performing complete medium designed and optimized for short term bone marrow culture. It provides a reliable and consistent number of mitotic cells suitable for karyotyping for more accurate cytogenetic analysis. Chang Marrow™ is an excellent medium to choose when culturing bone marrow aspirates.