Osteoinductivity Validation Summary of VAL-305 and VAL-435

Prepared by DCI Donor Services Tissue Bank

OI Test Methodology

Historically, DCI has performed osteoinductivity testing under the in vitro alkaline phosphatase test method developed by Dr. Bo Han.[1] Osteoinductivity testing was performed by Tissue Engineering Lab in the Department of Surgery at the University of Southern California. This lab processes under their In vitro assay of osteoinductive growth factors preserved in DBM by C2C12 Differentiation Assay. A summary of this Assay is described below:

> "C2C12, a pluriopotent myoblast cell line, has been found to undergo osteogenic differentiation and complete suppression of its myoblastic phenotype by suitable inductive signals [...] Active bone morphogenic proteins in DBM can simulate osteogenic differentiation of pre-myoblasts by inducing ALP activity." (Han et al.) In vitro assay quantifies the ALP activity. ALP activity is then converted into an OI "score":

$$OI = \frac{(ALPtest - ALPneg)}{\frac{ALPneg}{Protein}}$$

It was also shown that there is a strong correlation between in vitro cell differentiation and in vivo osteoinduction by implantation in a nude rat. Based on the vitro/vivo correlation studies, Han et al. recommend that a DBM with an OI over 0.2 of the negative baseline should be considered osteoinductively positive.

After accumulating running historic data of OI scores achieved by DCI's demineralized bone product line, DCI completed a retrospective validation of osteoinductivity processed under DCI Standard Operating Procedures.

In Vitro OI Results

Between the dates of 4/12/16-1/24/2017 in vitro osteoinductivity testing was performed on each lot of DCI's demineralized bone products as a release criteria. 100% of the donors processed, for a total of 76 donors, achieved a passing OI score over 0.2. Over a total of 84 consecutive samples, average OI test scores were 5.02 +/- 3.14.

In Vivo OI Results

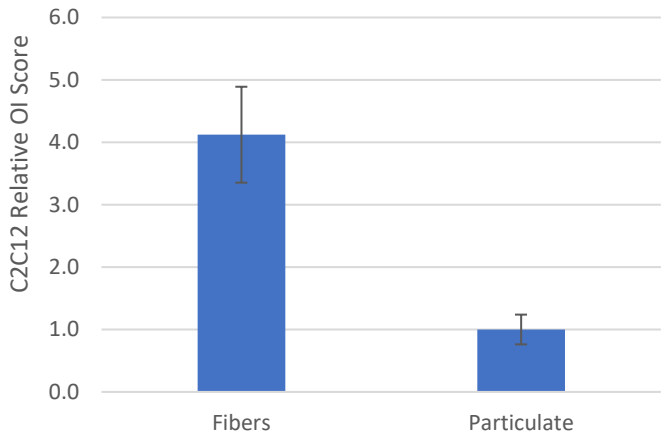
Following the accumulation of this in vitro data, matched samples from 4 donors included in the data set mentioned above were evaluated by in vivo osteoinductivity testing. The test was based on a method now considered the gold standard in the industry.[2]

Briefly, the samples of demineralized bone matrix were implanted into a muscle pouch of skeletally mature athymic rats. Following four weeks, the implant site was histologically evaluated for evidence of ectopic bone formation using a pre-determined histological scoring

matrix. Elements of new bone formation were observed in 6 out of 6 implant sites. The evaluated samples met the histological criteria for evidence of osteoinduction, thereby demonstrating osteoinduction potential in the intramuscular implant site using the male athymic nude rat model. This branch of the validation was to further correlate the in vivo and in vitro test results.

Comparative OI Results

Furthermore, a second retrospective validation was performed to analyze the different osteoinductivity scores between demineralized cortical powder and demineralized cortical fibers. Theoretically as the OI score increases, osteoinductivity increases as well. However, there have been no correlation studies to statistically illustrate the direct relationship between the in vitro OI score and in vivo osteoinductivity (besides the binary yes/no). Through analysis of historic fiber OI scores, we showed that fibers possessed a 4.1 fold higher OI score than particulate from the same donors as detailed in the chart below.



Summary

These data demonstrate the DCI Donor Services Tissue Bank's current manufacturing protocols produce demineralized cortical allografts with a high confidence of obtaining passing OI test results. The data also demonstrated DCI Donor Services Tissue Bank's current manufacturing protocols produce demineralized cortical allografts with positive in vivo OI test results. Results for future osteoinductivity testing will continue to be monitored, but will not be performed per donor as a release criteria, and appropriate actions will be taken as needed at the discretion of the DCI Donor Services Tissue Bank management team. All results are inclusive of DCI's Reficio® product line. Further data held on file at DCI Donor Services.

Additional References

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