

# Chordoma Foundation Cell Line Validation

## MUG-CC1

Cell Line Phenotype and Expression  
Analysis Report  
November 10, 2015

# Cell Line Receiving

| Format Received | Date Received | Condition     | Quantity | Passage | Initial Cell Count | Initial Cell Viability |
|-----------------|---------------|---------------|----------|---------|--------------------|------------------------|
| Flask (T25)     | June 10, 2015 | 50% confluent | 1        | p. 16   | N/A                | N/A                    |

## Growth Conditions

Media:

1:4 RPMI/IMDM + 10% FBS

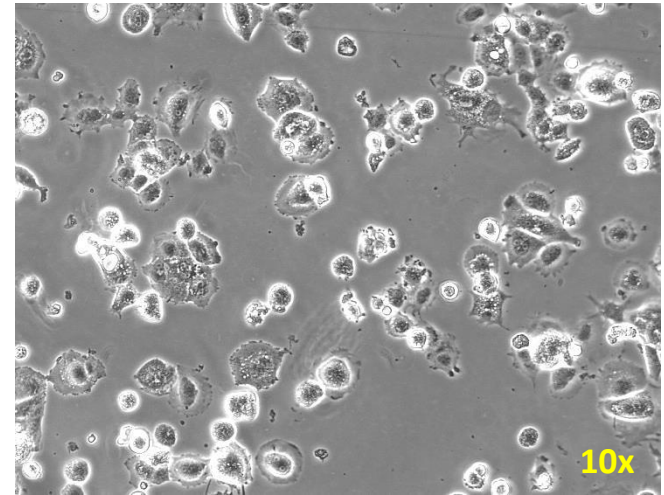
+ glut + ITS+ Pen/Strep

→ Passage when ~80-90% confluent (1:2, 1:3)

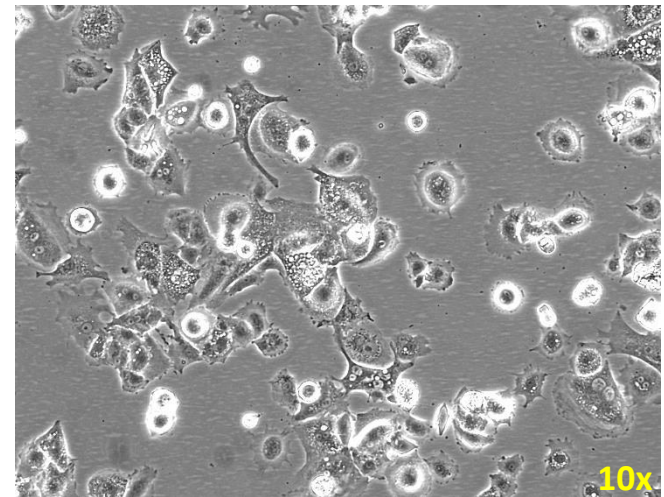
→ Change media every 2-3 days

## Phase Contrast Image Review

Cells arrived live, in 1 T25 closed-cap flask. Were semi-sparse (~50% confluent). They were visibly clear of contamination. They had to be moved, 24 hours after arrival, into a flask with a filtered cap.



Date Received (06/10/2015)

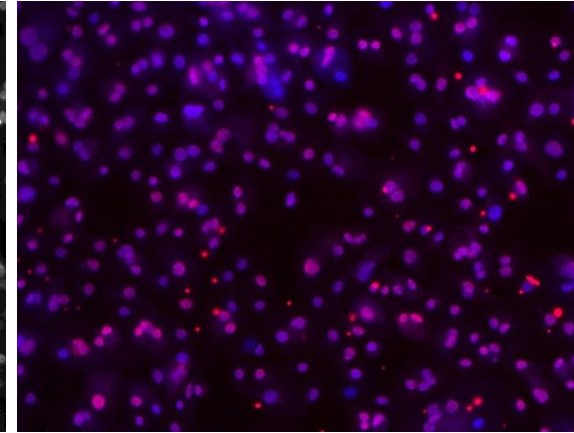
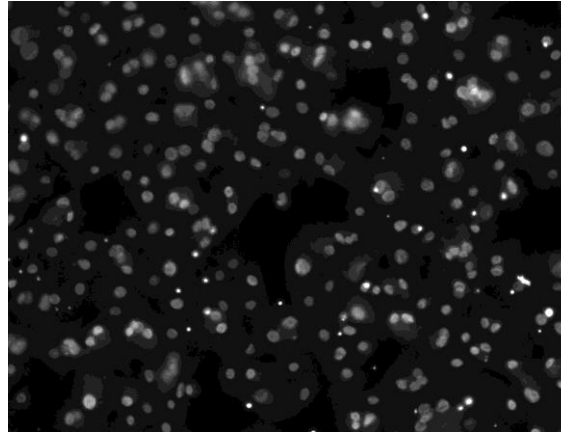
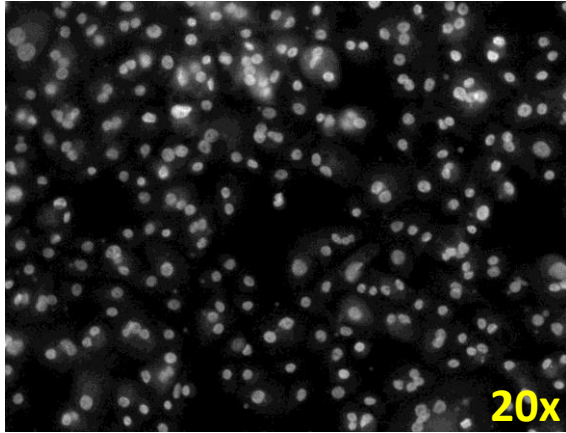


7 days post initial in-house passage  
(06/18/2015)

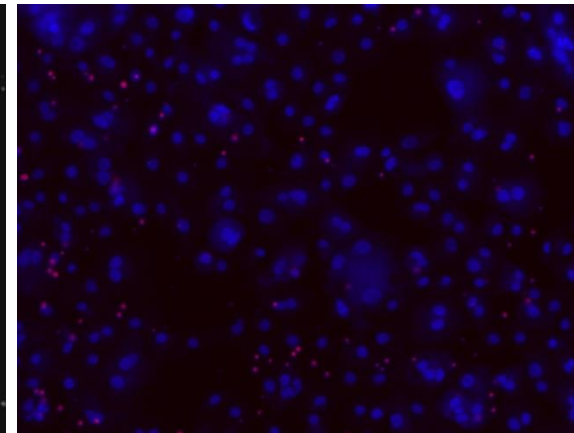
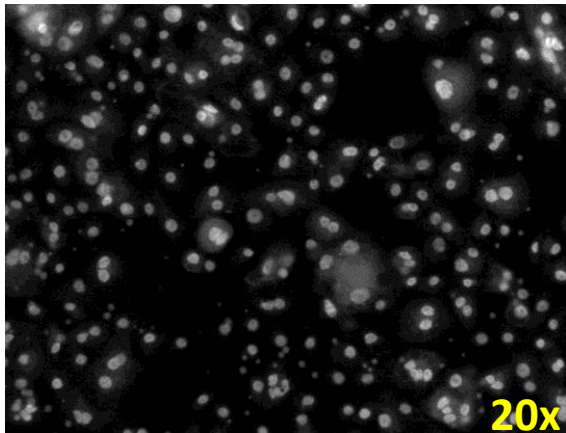
# Cell Line Immunofluorescence Validation

**MUG-CC1 p.22** versus Secondary Only Negative Control

MUG-CC1  
Cells



Secondary  
Only  
Negative  
Control



Hoechst Nuclear Stain

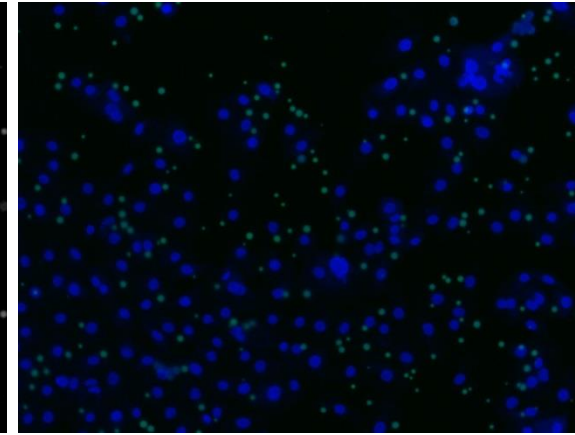
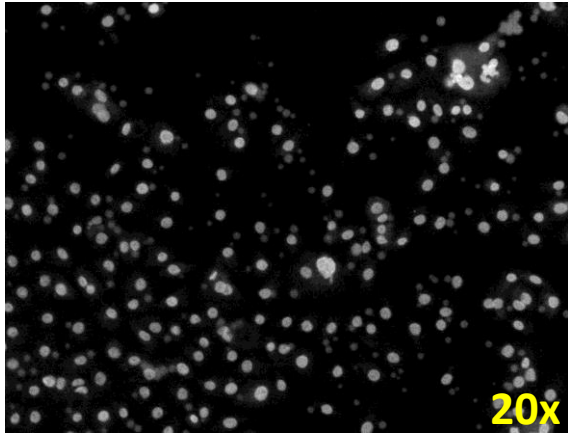
Anti-Brachyury Antibody

Color Composite

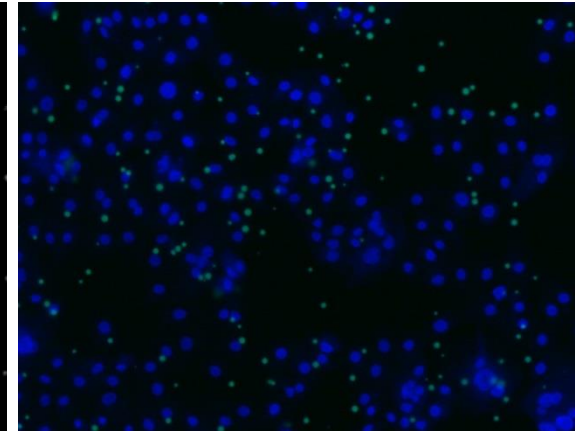
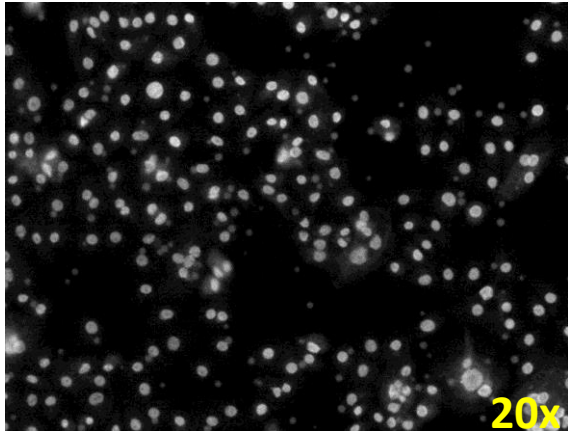
# Cell Line Immunofluorescence Validation

**MUG-CC1** p.22 versus Secondary Only Negative Control

MUG-CC1  
Cells



Secondary  
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Hoechst Nuclear Stain

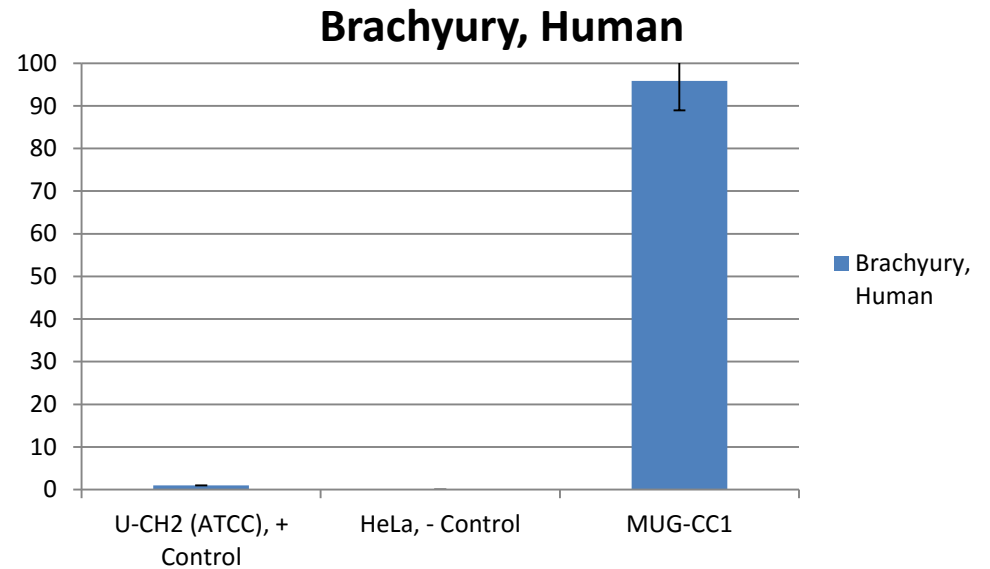
Anti-CD24 Antibody

Color Composite

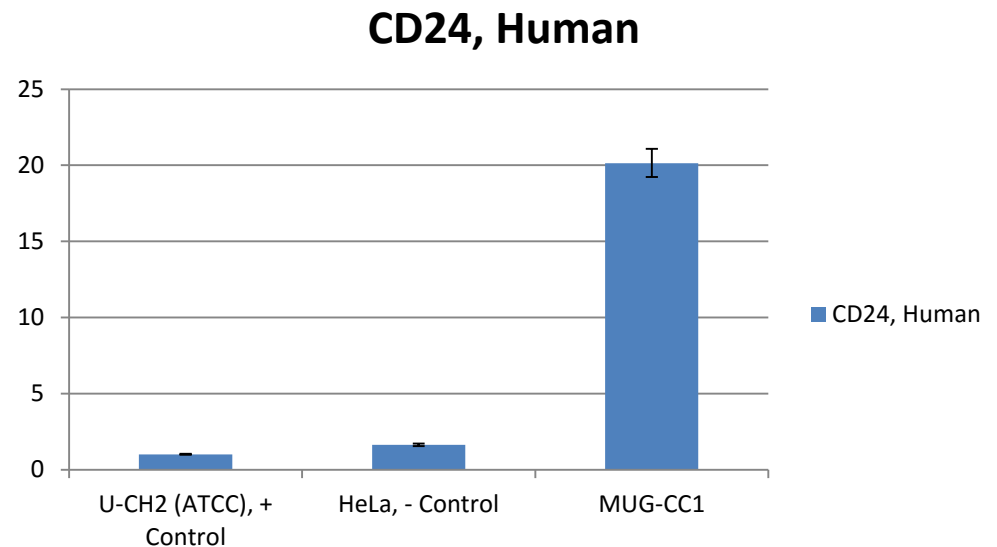
# Cell Line Validation Service

Relative quantification of Brachyury and CD24 in MUG-CC1 Chordoma cell line

| Sample Name             | Brachyury, Human | Neg Error | Pos Error |
|-------------------------|------------------|-----------|-----------|
| U-CH2 (ATCC), + Control | 1                | 0.025434  | 0.026098  |
| HeLa, - Control         | 0.012224         | 0.000844  | 0.000906  |
| MUG-CC1                 | 95.84546         | 6.937544  | 7.478885  |



| Sample                  | CD24, Human | Neg. Error | Pos. Error |
|-------------------------|-------------|------------|------------|
| U-CH2 (ATCC), + Control | 1           | 0.03154    | 0.032568   |
| HeLa, - Control         | 1.631788    | 0.086057   | 0.090848   |
| MUG-CC1                 | 20.13907    | 0.905041   | 0.947627   |



Tables and associated graphs depict relative quantification of brachyury (top table and graph) and CD24 (bottom table and graph) gene expression/RNA in chordoma cell samples. Gene expression across all assessed lines is set relative to the positive control sample, U-CH1, which is set at 1. The X-axis represents cell lines assessed and the Y-axis represents gene expression relative to positive control.

# Cell Line Validation Results

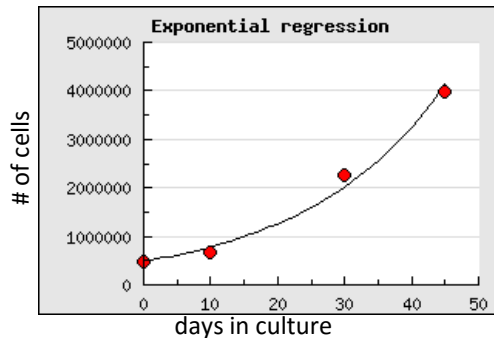
Results summary report of MUG-CC1

| TEST           | SPECIFICATION                 | RESULTS                 |
|----------------|-------------------------------|-------------------------|
| Cell Growth    | Immortalized                  | Doubling time = 15 days |
| STR Analysis   | Human, unique                 | Pass                    |
| IF Validation  | Signal in nucleus             | Pass                    |
| PCR Validation | Expressing Brachyury and CD24 | Pass                    |

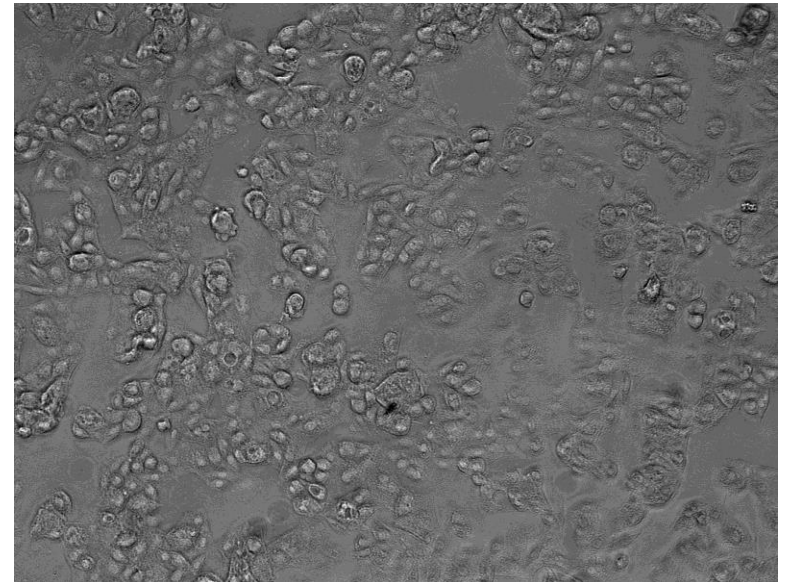
Cell lot generated

|                |                     |
|----------------|---------------------|
| Stock Lot#     | 1019-114            |
| Cells per vial | 1.0x10 <sup>6</sup> |
| Lot Viability  | 98%                 |
| Passages       | p.26                |

**Cell Line Growth:** Cell doubling time= 15 days



Cell growth rates were calculated from an actively growing culture for four passages. Growth rates will likely be slower when calculated from a fresh thaw.



MUG-CC1 Vala Cell Lot 1019-114, taken before validation IF labelling.