Data Sheet

OSUMMER.3 Mouse NRAS-Mutant Melanoma Cell Line

Cancer Cell Line

SCC447

Pack Size ≥ 1x10^6 viable cells/vial

Store at: Liquid nitrogen

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for Human or Animal Consumption.

Background

Melanoma skin cancer has had a growing incidence in the modern era. This type of skin cancer develops from melanocytes, a cell type found in the skin that is involved in melanin production which influences skin pigmentation. While skin cancers are the most common type of cancers, melanoma is quite rare and only accounts for about 1% of skin cancer cases.[1] However, melanoma is considered a very dangerous form of skin cancer due to its capability to spread if not caught in early stages. Early-stage melanoma can be cured by resection, but fewer treatment options are available for patients with metastatic disease. Development of resistance to targeted and immunotherapies, as well as toxicity are major problems. Along with sun exposure, sporadic genetic mutations have been associated with melanomas.[2] Immunotherapy treatments targeting these genes or pathways have been proven to have significant benefits in melanoma patients by improving general response and survival.

The OSUMMER (Ohio State University and Moffitt Melanoma Exposed to Radiation) cell lines fill a previously unfilled gap in melanoma biology. The OSUMMER cell lines are syngeneic to C57BL/6 laboratory mice and have genetic profiles that are similar to human tumors, making them responsive to immunotherapy treatments. These NRAS-Mutant cell lines can be used to discover and address potential immunotherapies in NRAS-mutant human melanomas which make up an estimated 15-25% of human melanomas. These cell lines also enable in vivo testing of immunotherapies with a mouse model.

OSUMMER.3 NRAS mouse melanoma cell line was derived from a female TN mouse exposed to UVA and UVB radiation. OSUMMER.3 carries the specific NRAS mutation Q61R and can form tumors in vivo without Matrigel® injection. WES revealed high SNV (Single Nucleotide variants) and low CNA (Copy Number Alterations) burden, as well as the presence of UV signature mutations. OSUMMER.3 cells express the melanoma markers, PMEL and MelanA, and exhibit robust growth in culture.

Source

OSUMMER.3 was derived from melanoma induced in TN (Tyr::CreER) mice exposed to UVA or UVB radiation on postnatal day 3.[3]

Short Tandem Repeat

M18-3: 16	M7-1: 26.2, 27.2	M15-3: 22.3	M12-1: 17
M4-2: 20.3	M1-1: 16	M6-4: 18	M5-5: 18, 19
M6-7: 15	M3-2: 14, 15	M11-2: 16	MX-1: 27
M19-2: 13	M8-1: 16	M17-2: 15	M13-1: 17
M1-2: 19	M2-1: 9		

Cancer cell lines are inherently genetically unstable. Instability may arise in the form of loss of heterozygosity of alleles at one or more genetic sites with increased passages.

Quality Control Testing

- Each vial contains ≥ $1X10^6$ viable cells.
- Cells are tested negative for infectious diseases by a Mouse Essential CLEAR Panel by Charles River Animal Diagnostic Services.
- Cells are verified to be of mouse origin and negative for inter-species contamination from human, rat, Chinese hamster, Golden Syrian hamster, and Non-human Primate (NHP) as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services
- Cells are negative for mycoplasma contamination.

Storage and Handling

OSUMMER.3 cells should be stored in liquid nitrogen. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting functionality.

Representative Data

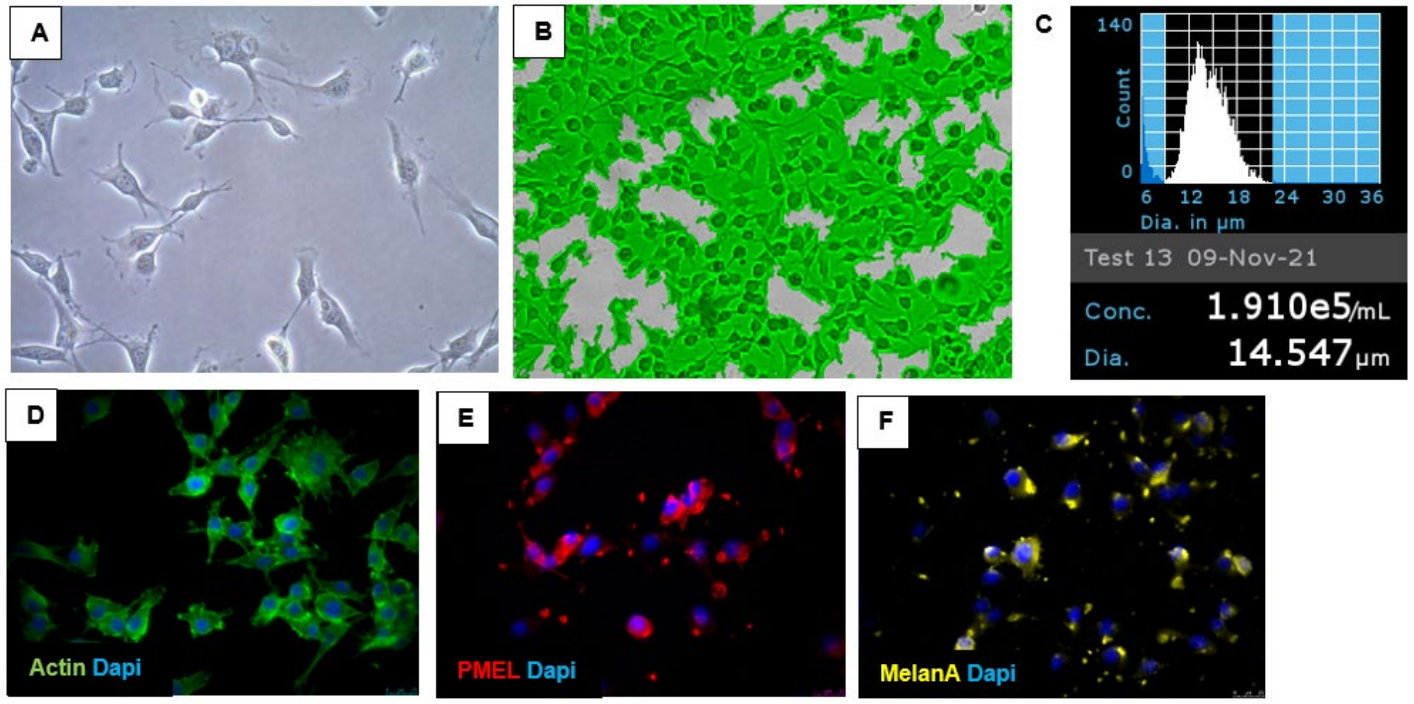

Figure 1. Bright-field image of OSUMMER.3 cells one day after thaw in a T75 flask (**A**). Cell confluency (80%) was assessed throughout the culture using MilliCell® Digital Cell Imager (**B**, Cat. No. MDCI 10000). Cell counting was performed using Scepter™ 3.0 handheld automated cell counter using 60 µm sensor tips (**C**, PHCC360KIT). OSUMMER.3 cells express actin (**D**) and the melanoma markers, PMEL (**E**) and MelanA (**F**).

Protocols

Thawing Cells

1. Do not thaw the cells until the recommended medium is on hand. Cells can grow on normal tissue cultureware surfaces without any additional coating.

   Cells are thawed and expanded in OSUMMER Expansion Medium comprised of DMEM-High Glucose medium (Cat. No. D5796) with 10% FBS (Cat. No. ES-009-B), 2 mM L-Glutamine (Cat. No. TMS-002-C), and 1X penicillin/streptomycin (Cat. No. TMS-AB2-C, optional).
2. Remove the vial of frozen OSUMMER.3 cells from liquid nitrogen and incubate in a 37 °C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.

   IMPORTANT: Do not vortex the cells.
3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
4. In a laminar flow hood, use a 1- or 2-mL pipette to transfer the cells to a sterile 50 mL conical tube. Be careful not to introduce any bubbles during the transfer process.
5. Using a 10 mL pipette, slowly add dropwise 9 mL of OSUMMER Expansion Medium (Step 1 above) to the 15 mL conical tube.

   IMPORTANT: Do not add the entire volume of media all at once to the cells. This may result in decreased cell viability due to osmotic shock.
6. Gently mix the cell suspension by slowly pipetting up and down twice. Be careful not to introduce any bubbles.

   IMPORTANT: Do not vortex the cells.
7. Centrifuge the tube at 300 *x g* for 2-3 minutes to pellet the cells.

8. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in 15 mL of OSUMMER Expansion Medium.
10. Transfer the cell mixture to a T75 tissue culture flask.
11. Incubate the cells at 37 °C in a humidified incubator with 5% CO_2.

Subculturing Cells

1. OSUMMER.3 cells should be passaged at ~80-85% confluency. Do not allow the cells to grow over 85% confluency.
2. Carefully remove the medium from the T75 tissue culture flask containing the 80% confluent layer of OSUMMER.3 cells. Rinse the flask with 10 mL 1X PBS. Aspirate after the rinse.
3. Apply 3-5 mL of Accutase™ or Trypsin/EDTA solution and incubate in a 37 °C incubator for 3-5 minutes.
4. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
5. Add 8 mL of OSUMMER Expansion Medium to the plate.
6. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
7. Centrifuge the tube at 300 *x g* for 3-5 minutes to pellet the cells.
8. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
9. Apply 2-5 mL of OSUMMER Expansion Medium to the conical tube and resuspend the cells thoroughly.

   IMPORTANT: Do not vortex the cells.
10. Count the number of cells using a hemocytometer or a Scepter™ 3.0 handheld automated cell counter using 60 µm sensor tips.
11. Plate the cells to the desired density. Typical split ratio is 1:5 to 1:6. The medium should be replaced every other day.

Cryopreservation of Cells

OSUMMER.3 cells may be frozen in OSUMMER Expansion Medium and 10% DMSO using a Nalgene® slow freeze Mr. Frosty® container.

References

1. Nat Rev Dis Primers 2015, 1: 15003.
2. Lancet 2018, 392(10151): 971-984.
3. Life Sci Alliance 2021, 4(9): e202101135.

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