Development and Evaluation of ImmunoCell™ Growth Medium (Akron ICGM): Xeno-free cell expansion medium demonstrated to Support Superior Cell Activation, Viability, Transduction and Expansion

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ABSTRACT

Over the past decade, significant progress has been made in T cell-based immunotherapy. Naturally occurring or genetically engineered T-cells have been used to target cancer antigens in hematological malignancies and solid tumors. The approach has been proven to be promising and has been validated with recent clinical trial successes and FDA approvals of several Chimeric Antigen Receptor (CAR) T-cell therapy products. Current treatments require that tumor cytotoxic T-cells isolated from a patient or donor be modified, activated and expanded ex vivo prior to being re-infused back into the patient. Culture medium that can effectively support ex vivo activation, transduction, and expansion of isolated T-cells has been a point of focus for the industry for several years, with several T-cell media commercially available. Leveraging our expertise in the development and manufacture of ancillary products, we designed and evaluated several T-cell media formulations with the capacity to promote outsized rates of PBMC growth while maintaining cell viability (Akron ImmunoCell™ Growth Medium (Akron ICGM)). Here, we summarize data from our initial media development studies to the five (5) lead formulations that were selected for their favorable features (i.e. component quality, xeno-free) and their comparison with commercially available products. Akron's ICGM Formula C showed superior performance and was sent to several partners for evaluation on their cell therapy platforms using T-cells isolated from healthy donors. We report data generated by Cytiva, using Akron ICGM Formula to culture healthy donor-derived T-cells.

BACKGROUND

Early development efforts focused on generating media formulations with the capacity to expand primary cells ex vivo leveraged a basal medium (i.e. RPMI 1640) supplemented with various components, including up to 10% fetal bovine serum. In an effort to eliminate serum from cell culture media, Iscove's modified Dulbecco's medium (IMDM) was developed in the late 1970s, incorporating components such as human transferrin, complex lipids, and supplementing buffering capacity with HEPES. Recent developments have focused on further de-risking these ancillary materials, driving toward chemically-defined formulations where feasible, and ensuring the use of USP/EP-compliant materials where available. In addition to ensuring media manufacturing under cGMP compliance, providing the lot-to-lot consistency to streamline cell therapy manufacturing, the sterility assurance required for patient safety have also been points of focus for the industry. Akron Bio has been providing cytokines, growth factors, and human plasma-derived media supplements to the cell and gene therapy industry since 2006. Akron's products and services are now embedded in nearly 150 clinical trials and commercial programs in the US, Europe and Asia. Leveraging decades of experience in supporting cell therapy development with media supplements, Akron has broadened its portfolio of solutions by developing a novel expansion medium. Akron ICGM has been developed, produced, and evaluated for performance over time, with accelerated and long-term stability underway. We have evaluated product performance on model cell lines (inc. Jurkat T-cells, THP-1 cells and PBMC derived T-cells). To confirm the functionality of the top formulation, Cytiva tested the medium on its cell therapy platform using T-cells isolated from three healthy donors.

MATERIALS & METHODS

During initial media development, the effect of different supplements on the Base Media ability to promote Human Peripheral Blood Mononuclear Cells (PBMCs) derived T-cells growth was assessed by using the ViCell XR cell counter (Beckman Coulter) to obtain Viable cells concentration and Cell viability. All evaluation were performed on 24-well plates seeded at 250,000 cells/well. Lead ICGM (Formula C) was tested by an independent testing contract organization for cell proliferation on PBMCs, immortalized line of human T lymphocyte cell (Jurkat T-cells), and a monocyte isolated from peripheral blood from an acute monocytic leukemia patient (THP-1 cells). Cells were seeded in a 96-well plate at 300,000 cells/well (PBMC), 20,000 cells/well (Jurkat T-cells) and 30,000 cells/well (THP-1). Plates were incubated at 37ºC. CellTiter 96 AQueous One Solution reagent from Promega was used determine the number of viable cells in proliferation or cytotoxic assays.

Healthy donor's T-cells were purified from fresh apheresis and stimulated by MACS® GMP T Cell TransAct™ Large Scale CR/GMP (Miltenyi Biotec) and expanded in ICGM Formula C using G-Rex production platform. Cell count was performed using automated cell counter NucleoCounter® NC-200. Memory phenotype was assessed using CD62L and CD45RO. Activation/Exhaustion was evaluated using CD25 and CD279. Transduction efficiency was determined by measuring conjugated recombinant human CD19 protein.

RESULTS

EFFECT OF BASAL MEDIA SUPPLEMENTATION

Components	Basal Media Supplementation					
	A	B	C	D	E	F
Akron IL-2 100 Units/mL	+	+	+	+	+	+
Iron Source #1	+	+	+	-	-	-
Iron Source #2	-	-	-	+	+	+
Human Serum Albumin (HSA)	-	2.5X	-	-	2.5X	-
Polyvinyl Alcohol (PVA) - 0.020%	-	-	2X	-	-	2X

Components	Basal Media Supplementation					
	A	B	C	D	E	F
Akron IL-2 100 Units/mL	+	+	+	+	+	+
Iron Source #1	+	+	+	+	+	+
Human Serum Albumin (HSA)	0	2.5X	2.0X	1.0X	0.5X	-
Polyvinyl Alcohol (PVA)	-	-	-	-	-	1.0X

Components	Basal Media Supplementation						
	A	B	C	D	E	F	
Akron IL-2 100 Units/mL	+	+	+	+			
Iron Source #1	+	+	+	+			
Human Serum Albumin (HSA)	2.5X	2.5X	2.5X	2.5X			
Antioxidant Element #1	-	+	-	+			Commercial Media B
Divalent Salt	-	-	+	+			

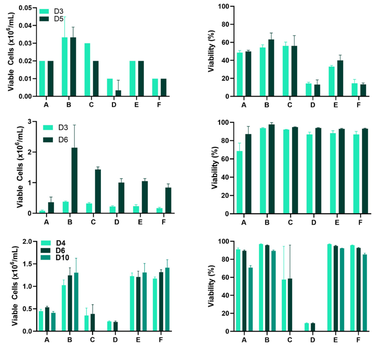


Figure 1: Basal media was supplemented with two sources of iron, Akron's HSA or PVA. All formulations were supplemented with Akron's IL-2 at 100 Units/mL prior to use. Cell growth was measured by cell counting on Day 3 and Day 5 post-seeding. Error bars represent SD from triplicate wells.

Figure 2: Basal media was supplemented with Iron source #1, Akron's HSA at various concentrations or PVA as reference. All formulations were supplemented with Akron's IL-2 at 100 Units/mL prior to use. Cell growth was measured by cell counting on Day 3 and Day 6 post-seeding. Error bars represent SD from triplicate wells.

Figure 3: Basal media was supplemented with Iron source #1, Akron's HSA at 2.5X concentration, a Level 1 antioxidant, a source of divalent salt. All formulations were supplemented with Akron's IL-2 at 100 Units/mL prior to use. Two commercially available T-cell media were used for side-by-side comparison. Cell growth was measured by cell counting on Day 4, Day 6 and Day 10 post-seedingError bars represent SD from triplicate wells.

EFFECT OF LEVEL 2 ANTIOXIDANT SUPPLEMENTATION

Components	Basal Media Supplementation										
	A	B	C	D	E	F	G	H	I	J	K
Akron IL-2 100 Units/mL	+	+	+	+	+	+	+	+	+		
Iron Source #1	+	+	+	+	+	+	+	+	+		
Antioxidant Element #1	+	+	+	+	+	+	+	+	+		
Human Serum Albumin (HSA)	0	1.0X	1.0X	1.0X	1.0X	2.5X	2.5X	2.5X	2.5X		
Antioxidant Element #2	-	0	0.5X	1.0X	4.0X	0	0.5X	1.0X	4.0X		
										Commercial media A	Commercial media B

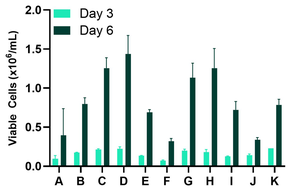
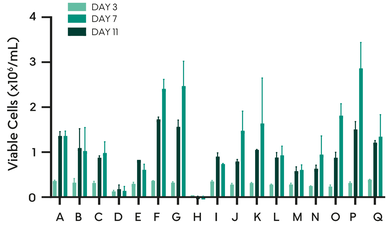


Figure 4: Basal media was supplemented with Iron source #1, a Level 1 antioxidant, Akron's HSA at 1.0X and 2.5X concentration, and a Level 2 antioxidant at various concentrations. All formulations were supplemented with Akron's IL-2 at 100 Units/mL prior to use. Cell growth was measured by cell counting on Day 3 and Day 6 post-seeding. Error bars represent SD from triplicate wells.

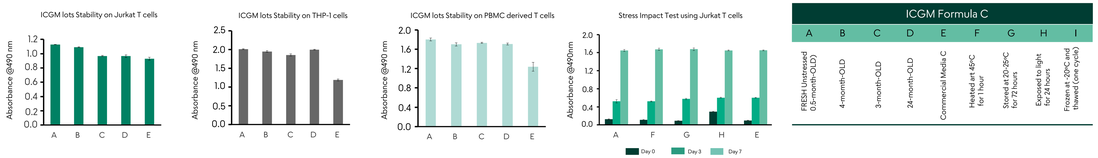
FINAL EVALUTATION OF LEAD MEDIUM FORMULATION (ICGM FORMULA)

COMPONENTS	ICGM Formula A			ICGM Formula B		ICGM Formula C		ICGM Formula D		ICGM Formula E						ICGM Formula C	
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
Akron IL-2 100 Units/mL	+	+	+	+	+	+	+	+	+	+	+					+	+
Basal Media Control	+	-	-	-	-	-	-	-	-	-	-					-	-
Minimum ICGM	-	+	+	+	+	+	+	+	+	+	+					+	+
Akron HSA	-	2.5X	2.5X	-	-	2.5X	2.5X	-	-	-	-					2.5X	2.5X
Polyvinyl Alcohol, PVA	-	-	-	2.0X	2.0X	-	-	1.0X	1.0X	-	-					-	-
Recombinant HSA	-	-	-	-	-	-	-	-	-	2.5X	2.5X					-	-
Antioxidant Element #2 (mM)				1.0X	1.0X	1.0X	1.0X	1.0X	1.0X	1.0X	1.0X					1.0X	1.0X
Akron viHu-AB Serum	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
Detergent	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
												Commercial Media A	Commercial Media A	Commercial Media B	Commercial Media B		

Figure 5: The optimal composition identified from the Basal media (Minimum ICGM) was supplemented with Akron's HSA at the optimal 2.5X concentration (ICGM Formula A); with Polyvinyl Alcohol, PVA at 2X concentration, in presence of the Level 2 antioxidant (ICGM Formula B); with Akron's HSA at the optimal 2.5X concentration in the presence of the level 2 antioxidant (ICGM Formula C); with PVA at 1.0X concentration and in the presence of the Level 2 antioxidant (ICGM Formula D); and with a commercially available Recombinant HSA at 2.5X concentration in the presence of the Level 2 antioxidant (ICGM Formula E). Each of the five formulas were further supplemented with Akron's virus inactivated Human AB serum (viHu-AB Serum) and Detergent to evaluate the impact on cell proliferation and cell health. Two commercially available T-cell media were used for side-by-side comparison. Cell growth was measured by cell counting on Day 3, Day 7 and Day 10 post-seeding. Error bars represent SD from triplicate wells.



ICGM FORMULA C STABILITY ASSESSMENT



ICGM Formula C								
A	B	C	D	E	F	G	H	I
FRESH Unstressed (0.5-month-OLD)	4-month-OLD	3-month-OLD	24-month-OLD	Commercial Media C	Heated at 45ºC for 1 hour	Stored at 20-26ºC for 72 hours	Exposed to light for 24 hours	Frozen at -20ºC and thawed (one cycle)

Figure 6: Lead ICGM Formula C supplemented with detergent was selected as the final Akron's T-cell media recipe. For stability assessment, archived lots were tested against a fresh lot. Furthermore, ICGM Formula C was subject to various stress conditions and performance was compared with unstressed medium. Cell expansion was measured using CellTiter 96 AQueous One Solution reagent from Promega. Error bars represent SD from triplicate wells.

ICGM FORMULA C PERFORMANCE WITH FRESH APHERESIS FROM HEALTHY DONORS

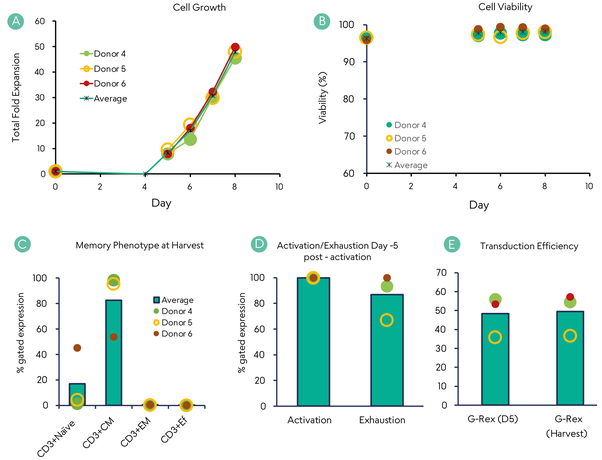


Figure 7: Purified T cells from healthy donors were, stimulated and expanded in ICGM Formula C using G-Rex production platform. Cells expansion (A) and cell viability (B) were with an NC200. Activated cells memory phenotype was assessed at harvest using CD62L and CD45RO(C). Activation/Exhaustion were evaluated at Day-5 post-activation using CD25 and CD279, respectively (D). Activated cells were transduced with anti-CD19 lentiviral Vector at MOI of 2.5. Transduction efficiency was evaluated at Day-5 post-activation and on harvested cell at Day-8, by measuring conjugated recombinant human CD19 protein (E). CD3+ CM = CD3+ central memory (CD45RO+, CD62L+); CD3+EM = Effector Memory (CD45RO+, CD62L-); CD3+ Ef = CD3+ (CD45RO-, CD62L+).

DISCUSSION

Optimized growth medium is essential for the large-scale expansion of patient-derived T-cell therapies and is critical to the success of adoptive cell therapy. We developed a novel T-cell medium that promotes the expansion of T-cells in vitro and has proven to be stable for up to 24 months post-manufacturing. The ICGM Formula C was compared to multiple commercially available T-cell medium and demonstrated an equivalent or better performance. We have shown that ICGM Formula C maintained cell growth function when subject to various stress such as, light exposure, elevated temperature and freeze-thaw cycle. Moreover, ICGM Formula C demonstrated its efficacy with the expansion of apheresis isolated T-cells. The donor derived T-cell evaluation was performed in partnership with our partner Cytiva-Cell Therapy division. Results obtained at small scale on the G-Rex platform showed that activated T-cells can be expanded up to 50-fold post-activation with minimal variability from one donor to the other. The immune-phenotype of the isolated cells is essential to the success of the treatment. Data showed that memory phenotypes of cells expanded in ICGM Formula C is maintained and that the % of transduced cells is around 50%.

REFERENCES/ACKNOWLEDGEMENTS

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