Cell therapy

The Nunclon Supra surface treatment enables serum- and coating-free culturing of mesenchymal stromal cells

Potential applications in cell therapy research

Introduction

By virtue of their pluripotency, stem cells are excellent resources in regenerative medicine. Stem cell therapy, in which stem cells are used for disease treatment or tissue repair, is an active area of research [1]. Treatment of many chronic diseases with mesenchymal stromal cells (MSCs) has yielded promising results for inducing tissue repair and regeneration [2,3]. The use of human MSCs (hMSCs) to treat various diseases is being investigated in more than 900 clinical trials [4]. Fetal bovine serum (FBS) is routinely used for culturing and expansion of MSCs in the laboratory, but the use of FBS carries the risk of xeno-immunity and zoonotic transmission for patients who receive MSCs for therapeutic purposes. For this reason, chemically defined xeno-free and serum-free media are used to culture therapeutic stem cells. However, culturing with serum-free media requires the use of extracellular matrix (ECM) proteins to help cells attach to conventional treated polystyrene surfaces. ECM proteins are rich in immunogenic molecules that can trigger an uncontrolled immune response and affect graft integration.

To circumvent these problems, we have introduced a line of cell culture plastics with Thermo Scientific™ Nunclon™ Supra™ surface treatment. The Nunclon Supra surface is superior for the growth of MSCs under serum-free conditions and does not require an ECM protein coating. The Nunclon Supra surface is generated by exposing molded plastic to a proprietary energy source that renders the surface more hydrophilic by increasing the concentration of hydroxyl groups. Anchorage-dependent cells are able to attach to the hydrophilic surface and become confluent.

Three types of hMSCs were tested on the Nunclon Supra surface: adipose tissue–derived stem cells (ADSCs), bone marrow–derived MSCs (BMMSCs), and umbilical cord–derived MSCs (WJMSCs). All MSCs grown on the Nunclon Supra surface retained their morphology, phenotype, and trilineage differentiation potential better than those grown on conventional polystyrene surfaces coated with ECM proteins. Nunclon Supra culture plastics are thus promising alternatives for growing hMSCs for potential cell therapy research applications.

Results

Human MSCs grown on the Nunclon Supra surface retain their morphology and health

Human MSCs cultured in serum-free, xeno-free medium can be grown on the Thermo Scientific™ Nunclon™ Delta surface after it is coated with Gibco™ CELLstart™ Substrate. To evaluate the suitability of the Nunclon Supra surface for supporting hMSC growth in serum-free medium, hMSCs were seeded onto the uncoated Nunclon Delta surface, the Nunclon Delta surface coated with CELLstart Substrate, and the uncoated Nunclon Supra surface at the same seeding density and allowed to reach confluency. As shown in Figure 1A, the morphology and confluency of all three types of hMSCs seeded on the uncoated Nunclon Supra surface were comparable to those of cells grown on the Nunclon Delta surface coated with CELLstart Substrate. As expected, the uncoated Nunclon Delta surface (Figure 1A, left) did not support cell growth. Although the doubling time of cells grown on the Nunclon Supra surface was longer than that of cells grown on the coated Nunclon Delta surface, the difference was not significant (Figure 1B). Cell viability (CV) was determined by the trypan blue exclusion method, and CV on the two surfaces was comparable (data not shown). These results demonstrate that the Nunclon Supra surface supports good hMSC attachment and proliferation without an ECM protein coating in serum-free conditions.

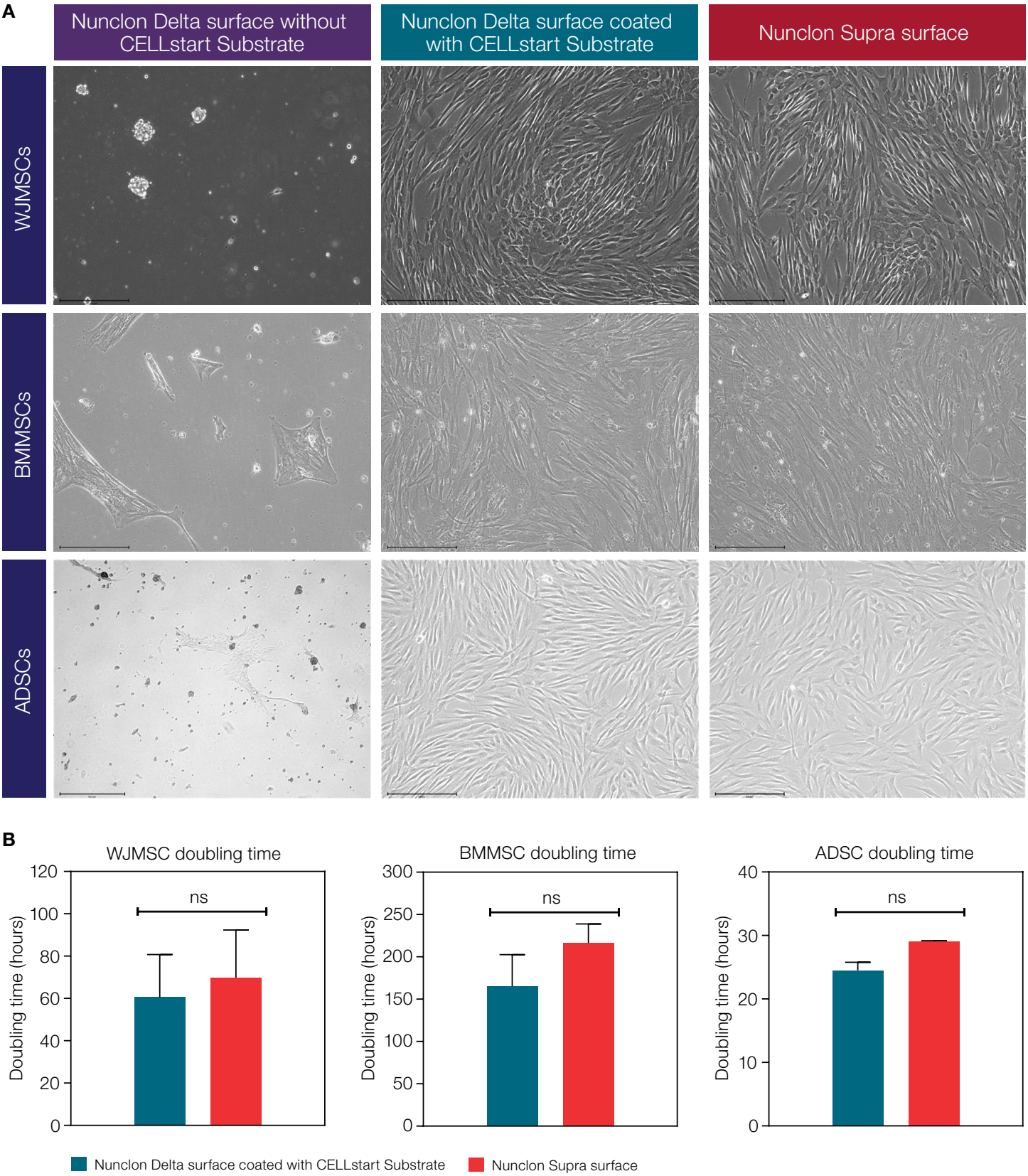

Figure 1. Growth and morphology of human mesenchymal stromal cells (hMSCs) on Nunclon Delta and Nunclon Supra surfaces. **(A)** Representative images showing the morphology of WJMSCs, BMMSCs, and ADSCs seeded on the uncoated Nunclon Delta surface, the Nunclon Delta surface coated with CELLstart Substrate, or the uncoated Nunclon Supra surface. Images were captured using the Invitrogen™ EVOS™ M7000 Imaging System with a 10x objective. Scale bar: 275 µm. **(B)** Mean doubling times of hMSCs grown for three passages on the Nunclon Delta surface coated with CELLstart Substrate or the uncoated Nunclon Supra surface (n = 3). The error bars represent the standard error of the mean, and ns indicates no significance in an unpaired t-test.

The Nunclon Supra surface preserves the hMSC phenotype

The immunophenotypic profiles of hMSCs grown on the Nunclon Supra surface were determined by testing a panel of surface markers via flow cytometry analysis. The cells were stained with marker antibodies and their corresponding isotype controls. Dead cells were excluded from analysis by staining them with appropriate viability dyes. Human MSCs grown on the Nunclon Supra surface showed positive expression of CD73, CD90, CD105, and CD44 along with negative expression of CD45, CD34, CD14, and CD79α. These marker profiles characterized the cells as MSCs [5]. Expression of these surface markers by cells grown on the Nunclon Supra surface was similar to expression by cells grown on the conventional Nunclon Delta surface coated with CELLstart Substrate. Figure 2 shows representative data for the phenotypic characterization of BMMSCs. ADSCs and WJMSCs showed comparable CD marker expression when grown on the two surfaces (data not shown).

Human MSCs grown on the Nunclon Supra surface retain their trilineage differentiation potential

Directed differentiation of hMSCs into desired cell types is critical for effective treatment strategies. To evaluate the functionality of hMSCs grown on the Nunclon Supra surface, the cells were differentiated into the characteristic adipocyte, osteocyte, and chondrocyte lineages. The cells were cultured in adipogenesis, chondrogenesis, or osteogenesis induction medium according to the respective manufacturer's instructions. Human MSCs grown on the Nunclon Supra surface successfully differentiated into all three cell lineages. Induction of WJMSCs into the chondrogenic lineage by the micromass method [6] resulted in deposition of proteoglycans that could be visualized with Thermo Scientific™ Alcian Blue 8GX stain (Figure 3A). Calcium deposition in osteocytes differentiated from BMMSCs was confirmed by positive staining with Thermo Scientific™ Alizarin Red dye (Figure 3B). ADSCs that differentiated into adipocytes synthesized intracellular lipid droplets, which was confirmed by staining them with Thermo Scientific™ Oil Red O dye (Figure 3C). Qualitative analysis revealed similar differentiation of cells on the Nunclon Delta surface coated with CELLstart Substrate. Overall, these data show that hMSCs grown on the uncoated Nunclon Supra surface retain their trilineage differentiation potential.

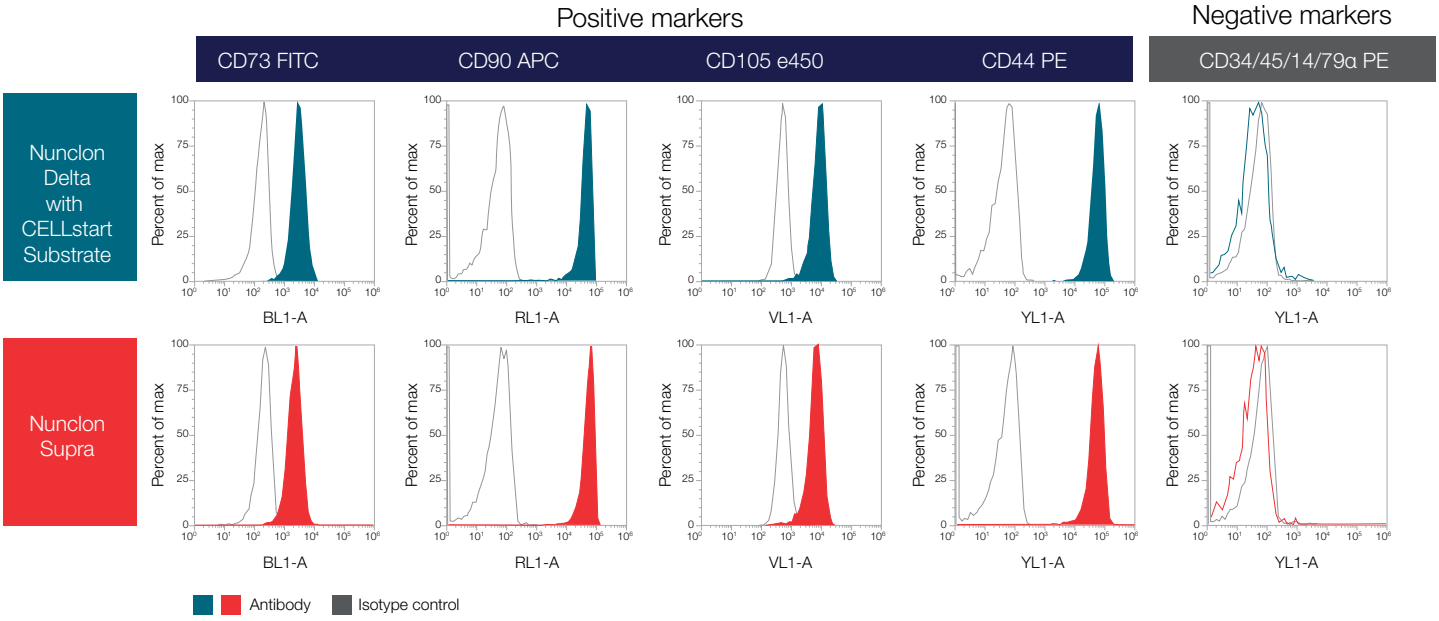

Figure 2. Comparison of the phenotypes of BMMSCs grown on Nunclon Delta and Nunclon Supra surfaces. The results of flow cytometry analysis are shown for cells grown on the uncoated Nunclon Supra surface or the Nunclon Delta surface coated with CELLstart Substrate. The cells showed positive expression of CD73, CD90, CD105, and CD44 along with negative expression of CD34, CD45, CD14, and CD79α. Ten thousand events were captured per sample. The x-axes represent the log fluorescence intensity. The y-axes represent the number of events as a percentage of the maximum.

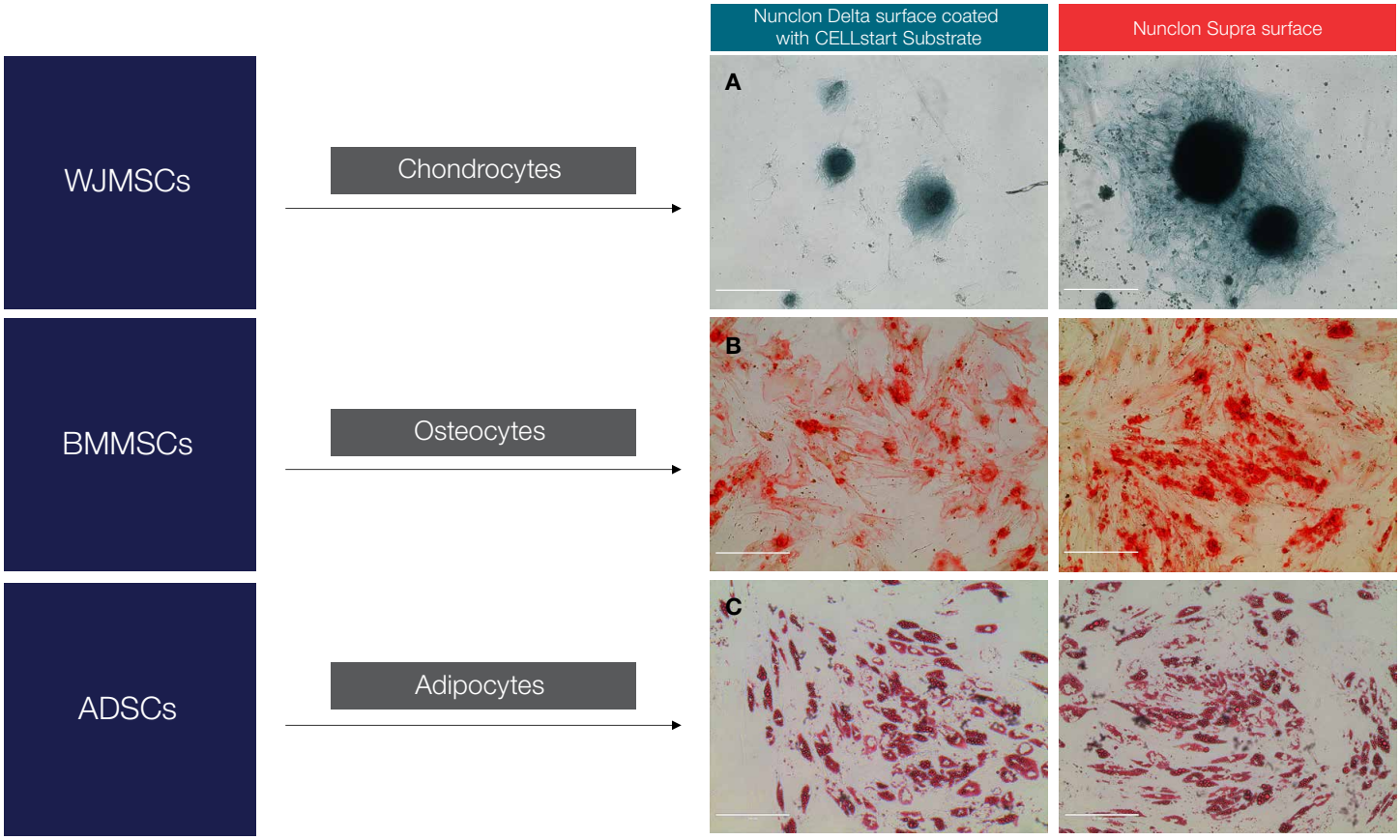

Figure 3. Differentiation of human mesenchymal stromal cells (hMSCs) on Nunclon Delta and Nunclon Supra surfaces. Representative images of **(A)** WJMSCs after differentiation into chondrocytes, **(B)** BMMSCs after differentiation into osteocytes, and **(C)** ADSCs after differentiation into adipocytes on the uncoated Nunclon Supra surface or the Nunclon Delta surface coated with CELLstart Substrate. Images were captured using the Invitrogen™ EVOS™ XL Core Imaging System with a 10x objective. Scale bar: 200 µm.

Conclusion

The use of hMSCs in translational research, particularly cell therapy research, is accelerating. The experimental data presented here demonstrate that the Nunclon Supra surface is suitable for supporting the growth and differentiation of hMSCs in serum-free and coating-free conditions. The Nunclon Supra surface can thus facilitate GMP culturing of these cells for cell therapy research applications.

Ordering information

	Product	Cat. No.
Cell culture plastics	Nunc Cell-Culture Treated Multidishes	140675, 142475
	Nunc 6-Well Plate with Nunclon Supra Surface Treatment	140680
	Nunc 12-Well Plate with Nunclon Supra Surface Treatment	140681
Cells, media, ECM products, and supplements	StemPro BM Mesenchymal Stem Cells	A15652
	StemPro Human Adipose-Derived Stem Cells	R7788115
	CELLstart Substrate	A1014201
	CTS DPBS (1X), without calcium chloride, without magnesium chloride	A1285601
	CTS DPBS, calcium, magnesium	A1285801
	CTS TrypLE Select Enzyme	A1285901
	StemPro MSC SFM XenoFree	A1067501
	Trypan Blue Solution, 0.4%	15250061
	Alcian Blue 8GX	J60122-22
	Oil Red O	A12989.14
	StemPro Adipogenesis Differentiation Kit	A1007001
	StemPro Chondrogenesis Differentiation Kit	A1007101
	StemPro Osteogenesis Differentiation Kit	A1007201
Antibodies and dyes	CD90 (Thy-1) Monoclonal Antibody (eBio5E10 (5E10)), APC, eBioscience	17-0909-42
	Mouse IgG1 Kappa Isotype Control (P3.6.2.8.1), APC, eBioscience	17-4714-82
	CD73 Monoclonal Antibody (AD2), FITC, eBioscience	11-0739-42
	Mouse IgG1 Kappa Isotype Control (P3.6.2.8.1), FITC, eBioscience	11-4714-81
	CD105 (Endoglin) Monoclonal Antibody (SN6), eFluor 450, eBioscience	48-1057-42
	Mouse IgG1 Kappa Isotype Control (P3.6.2.8.1), eFluor 450, eBioscience	48-4714-82
	CD44 Monoclonal Antibody (IM7), PE, eBioscience	12-0441-82
	CD34 Monoclonal Antibody (4H11), PE, eBioscience	12-0349-42
	CD45 Monoclonal Antibody (HI30), PE, eBioscience	12-0459-42
	CD14 Monoclonal Antibody (61D3), PE, eBioscience	12-0149-42
	CD79a Monoclonal Antibody (HM47), PE, eBioscience	12-0792-42
	Mouse IgG1 Kappa Isotype Control (P3.6.2.8.1), PE, eBioscience	12-4714-82
	eBioscience Fixable Viability Dye eFluor 450	65-0863-14
	eBioscience Fixable Viability Dye eFluor 780	65-0865-14
	UltraComp eBeads Compensation Beads	01-2222-42
Instruments	Countess 3 Automated Cell Counter	AMQAX2000
	EVOS XL Core Imaging System	AMEX1000
	EVOS M7000 Imaging System	AMF7000
	Attune NxT Flow Cytometer, blue/red/violet6/yellow	A29004

References

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