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Article type : Letter to the Editor

Journal to Consider Manuscript: *Allergy*

Manuscript Category: Letter to the Editor

Article Title: Interleukin-6: A novel biomarker for monoclonal antibody and chemotherapy-associated hypersensitivity confirms a cytokine-release syndrome phenotype-endotype association

Manuscript Word Count: 764

Number of References: 5

Number of Tables: 0

Number of Figures: 2

Number of Online Supplemental Data Tables: 6 (5 pages of material)

Authorship:

All authors met criteria for authorship. Study data were collated by BDJ, SSS, SH and DML. BDJ performed the data analysis and drafted the initial manuscript version which was reviewed by coauthors for intellectual content and approved of the final submitted version.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/ALL.14644](#)

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Acknowledgements: none

To the editor,

Interleukin-6(IL-6) is a pleotropic cytokine postulated to underlie a unique clinical phenotype observed in certain breakthrough reactions(BTRs) occurring during rapid drug desensitization(RDD) to chemotherapy and biologic agents.^{1,2} IL-6 is an endogenous pyrogen and proinflammatory mediator that plays a role in both innate and adaptive immunity.³ Under typical physiologic conditions, levels are relatively low, ranging from 1-5 pg/mL.³ In this study, we aimed to further explore IL-6 as a possible biomarker for BTRs seen during RDD to conventional chemotherapeutic or biologic agents, and determine the phenotypic constellation with which elevated levels may correlate. We hypothesized that IL-6 levels would rise during BTRs occurring in the context of RDD and furthermore, that elevated IL-6 levels would be seen in association with a characteristic phenotype of signs and symptoms that would distinguish and confirm a unique cytokine release syndrome etiology of drug hypersensitivity.

This was a retrospective study (IRB # protocol 2013P001672) in which we collected patients who experienced a breakthrough reaction during RDD to a chemotherapy or biologic agent from Oct 2015-March 2018 and had a serum IL-6 level drawn at the time of their reaction. The standard 3 bag 12 step protocol was the most commonly used protocol with few adapted changes.^{1,4} We collated baseline clinical features for the involved patients, as well as details surrounding the desensitization procedures, and breakthrough reaction signs and symptoms. This included both qualitative details surrounding the symptom complex as well as quantitative data including body temperature, heart rate, systolic blood pressure, respiratory rate, and oxygen saturation. Symptoms were further stratified into groupings by system. As desensitization is a multistep process and reactions can evolve over time, the entire

constellation of features observed during all steps of the procedure (up to and including the completion of period of observation) were included as part of the reaction phenotype.

We compared baseline to reaction serum IL-6 levels, and where possible, compared baseline to reaction serum tryptase levels. Using both a more liberal (<1.8 pg/mL) and conservative (<17.5 pg/mL) threshold for normal range of IL-6, we stratified the reactions to see if there was any difference in clinical features observed. These represented the normal threshold values for IL-6 which were modified by the processing laboratory during the study period. All analyses were performed in Microsoft excel software. Significance was predefined as $p<0.05$.

We identified 21 patients who had undergone a total of 38 RDD procedures during which a reaction occurred and serum IL-6 measured. Baseline patient characteristics, RDD treatment indication, reaction management, and desensitization outcome are outlined in Tables 1-3 (online supplement). Over 90% of patients were receiving treatment for cancer, with the majority undergoing RDD to a platin agent. Of the 38 reactions, 89.5% experienced cardiovascular symptoms, 73.7% endorsed constitutional symptoms, and 52.6% neuromuscular complaints. When the reaction features were subcategorized on the basis of normal or abnormal IL-6 (**Figure 1**), significant differences were noted with respect to fever of $>38^{\circ}\text{C}$ (IL6 >17.5 pg/mL 34.5% vs IL6 <17.5 pg/mL 0.0 %; $p=0.0008$) and with neuromuscular complaints (IL6 >17.5 pg/mL 65.5% vs IL6 <17.5 pg/mL 11.1%; $p=0.0042$). When the IL-6 data were reviewed using a normal threshold of <1.8 pg/mL, as 100% of reactions were noted to have elevated levels, the clinical feature frequency is the same as the overall total (Blue bars in Figure 1). Significant increases in body temperature, heart rate, and systolic blood pressure were also observed, though these changes did not linearly correlate with IL-6 levels. (Tables 4 and Table 5, online supplement).

IL-6 levels were significantly increased during the reactions at 627.8 ± 1007.5 pg/mL. Among these, baseline IL-6 levels for 19 reactions were compared, with a statistically significant mean difference from baseline to reaction noted (1066.8 ± 1257.7 ; $p=0.0008$). A comparable increase from basal to reaction serum tryptase was not observed (Table 6 online supplement; **Figure 2a-b**).

Our data demonstrate that IL-6 surges during BTRs are significant, and correlate with a clinical picture that includes thermal and hemodynamic changes, accompanied by prominent constitutional, cardiovascular, and neuromuscular symptoms. The principle limitations relate to our relatively small

sample size, and possibility that selection bias may impact on the widespread generalizability of our results. As RDD is thought to abrogate mediator release from mast cells,⁵ the source of IL-6 is likely from other immune cells, indicating that RDD induces drug tolerance by suppressing one, but not all pathways of immediate hypersensitivity reactions. Moreover, our data suggest IL-6 is an important biomarker that verifies a phenotype-endotype relationship supporting a cytokine release mechanism of immediate drug hypersensitivity. This may provide proof of concept for the use of anti-IL-6 therapies to further enhance the safety of drug administration and/or RDD.

References:

1. Isabwe GAC, Garcia Neuer M, de Las Vecillas Sanchez L, Lynch DM, Marquis K, Castells M. Hypersensitivity reactions to therapeutic monoclonal antibodies: Phenotypes and endotypes. *J Allergy Clin Immunol*. 2018;142(1):159-170.e152.
2. Silver J, Garcia-Neuer M, Lynch DM, Pasaoglu G, Sloane DE, Castells M. Endophenotyping Oxaliplatin Hypersensitivity: Personalizing Desensitization to the Atypical Platin. *J Allergy Clin Immunol Pract*. 2020;8(5):1668-1680.e1662.
3. Hunter CA, Jones SA. IL-6 as a keystone cytokine in health and disease. [Review]. *Nature Immunology*. 2015;1(5):448-457.

4. Sloane D, Govindarajulu U, Harrow-Mortelliti J, et al. Safety, Costs, and Efficacy of Rapid Drug Desensitizations to Chemotherapy and Monoclonal Antibodies. *J Allergy Clin Immunol Pract.* 2016;4(3):497-504.
5. Sancho-Serra Mdel C, Simarro M, Castells M. Rapid IgE desensitization is antigen specific and impairs early and late mast cell responses targeting FcεRI internalization. *Eur J Immunol.* 2011;41(4):1004-1013.

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Author Disclosures:

BDJ-none

SSS-none

SH-none

DML-none

MC-none

Funding Sources:

Ovations for the Cure Private Foundation Grant

Figure 1: Clinical phenotypes of breakthrough reactions stratified by normal vs elevated IL-6 level.

Figure 1 Legend:

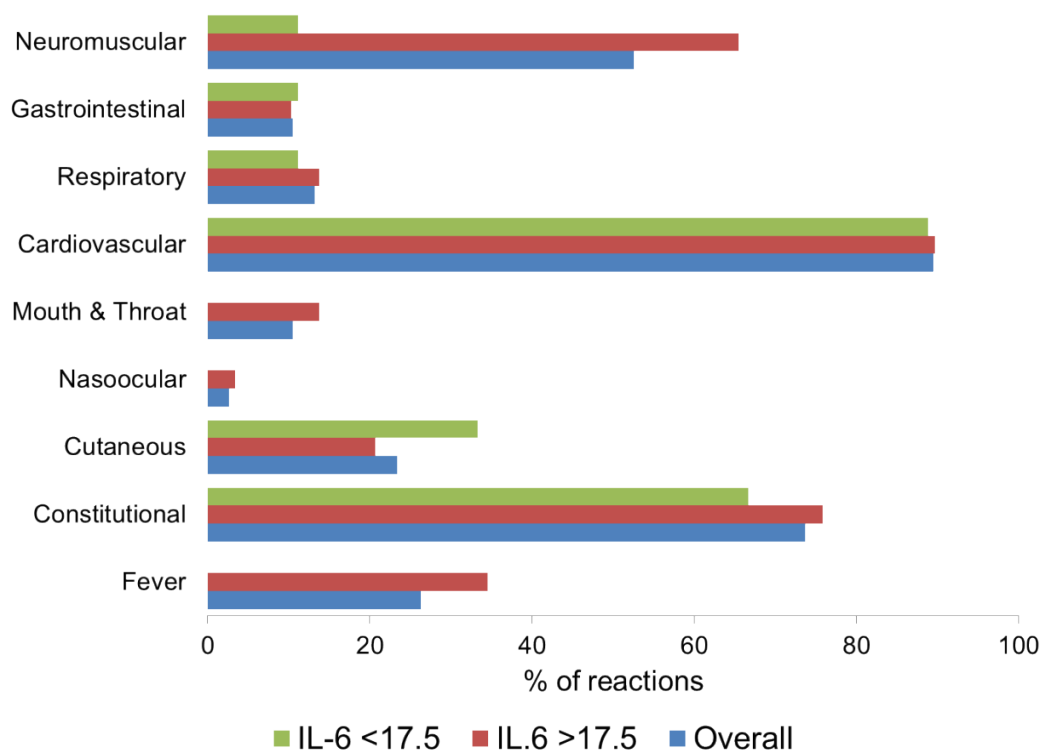
Clinical features occurring in the context of breakthrough reactions are organized by anatomical system and stratified by overall % of reactions containing the listed feature and sub-stratified by normal (IL-6<17.5 pg/mL) vs abnormal (IL-6>17.5 pg/mL). Of the 38 events, elevated IL-6 levels were seen in 29 reactions (76.3% of all reactions). All reactions in which fever was documented (>38 degrees Celsius) were associated with elevated IL-6 levels ($p = 0.0008$). Neuromuscular complaints were also significantly higher in reactions associated with an elevated IL-6 level (IL6>17.5 pg/mL 65.5% vs IL6<17.5 pg/mL 11.1%; $p = 0.0042$).

Figure 2: Comparison of baseline and reaction measurements for serum tryptase (A) and IL-6 (B)

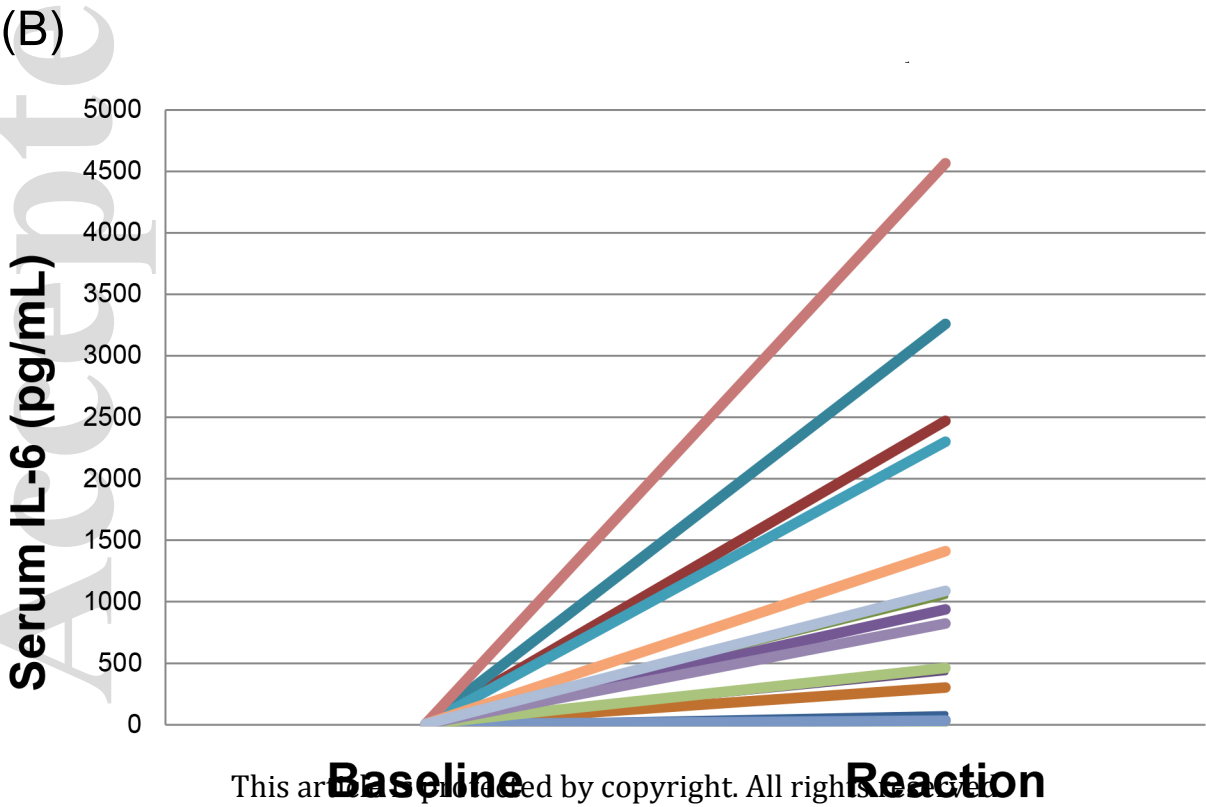
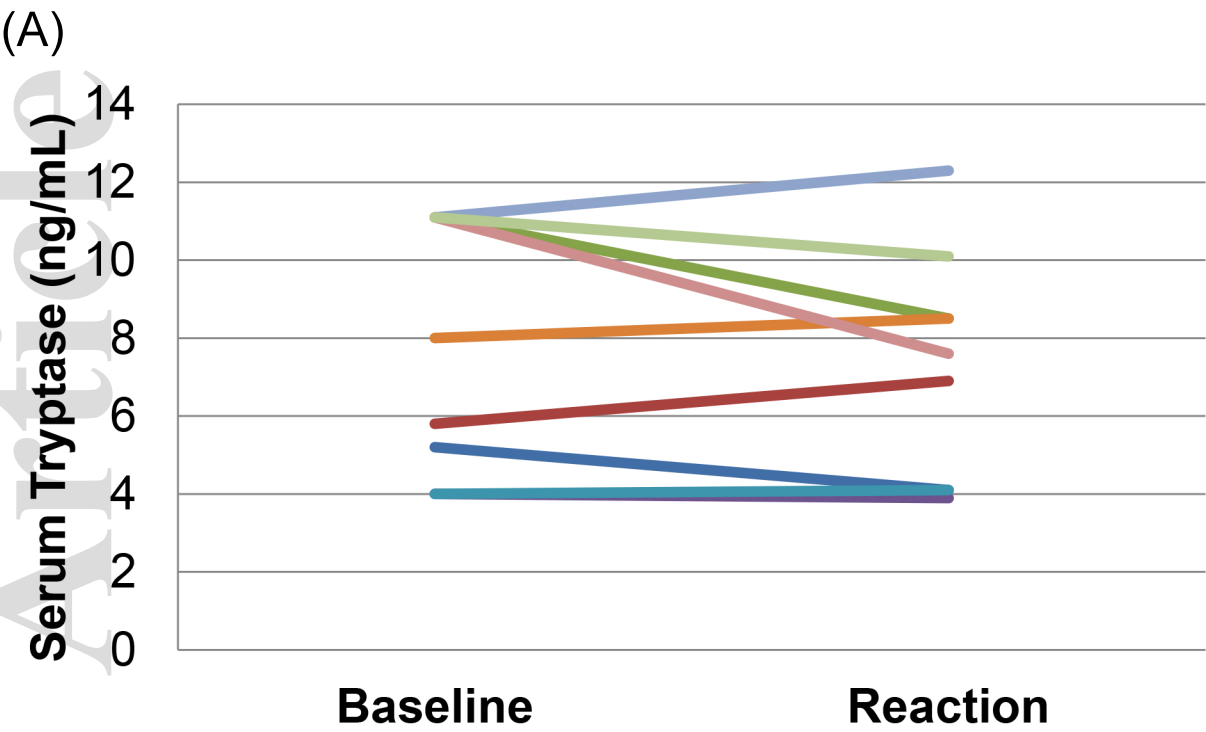
Figure 2 legend:

Accepted Article

These figures visually depict the change in serum levels of IL-6 and tryptase among the reactions where baseline and reaction samples were available for comparison. Significant rises in IL-6 (Figure 2B; $p=0.0008$), but not serum tryptase (2A), were observed during reactions as compared to baseline.



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Online Supplemental Materials

Table 1-Demographic characteristics of included patients

	N=21 patients (%)
Age (years)	
Max	83
Min	39
Gender	
Male	7 (33.3)
Female	14 (66.7)
Atopic history n (%)	
Any Atopy	17 (80.9)
Asthma	3 (14.3)
Allergic rhinitis	3 (14.3)
Drug allergy	13 (61.9)
Food allergy	1 (4.8)
Underlying disease (n, %)	
Cancer, all types	19 (90.5)
Gastrointestinal	13 (61.9)
Hematologic	5 (23.8)
Squamous cell head and neck	1 (4.8)
Autoimmune disease, all types	2 (9.5)
Rheumatoid Arthritis	2 (9.5)
History of Hypertension (n, %)	11 (52.4)
Initial Reaction Severity ¹	
Grade 1	1 (4.8)
Grade 2	17 (80.9)
Grade 3	3 (14.3)
Skin Testing Result	
Positive	10 (47.6)
Negative	6 (28.6)
Not performed	5 (23.8)

¹Grading as outlined by Brown et al.. *J Allergy Clin Immunol.* 2004;114(2):371-376.

10 **Table 2-Overall characteristics of the desensitisations during which reactions with measured IL-6**
 11 **occurred**

Factors	Total (n) =38 reactions
Drug Administered during desensitization (n, % total reactions)	24 (63.2)
Conventional chemotherapy	21 (55.3)
Oxaliplatin	3 (7.9)
Carboplatin	13 (34.2)
Biologic Therapy	6 (15.8)
Rituximab	3 (7.9)
Trastuzumab	2 (5.3)
Obinutuzumab	2 (5.3)
Pertuzumab	1 (2.6)
Antibody-drug conjugate therapy	1 (2.6)
Trastuzumab emtansine	
Location of Desensitization Procedure	
Outpatient infusion centre	37 (97.4)
Intensive Care unit	1 (2.6)
Protocol Utilized For Desensitization	
4 bag 16 step	5 (13.2)
3 bag 12 step	32 (84.2)
2 bag 8 step	1 (2.6)
Pretreatment Medications Administered	
H1 antihistamine	38 (100)
H2 antihistamine	38 (100)
Leukotriene antagonist	27 (71.1)
Steroids	13 (34.2)
Tylenol	2 (5.3)
NSAID	3 (7.9)
Acetylsalicylic acid	5 (13.2)
Anxiolytic	4 (10.5)
Meperidine	1 (2.6)
Gabapentin	4 (10.5)

12 ¹Nonsteroidal anti-inflammatory drug

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20 **Table 3-Reaction Management and Desensitization outcomes**

Parameter	38 total desensitizations N (%)
Reaction Treatments Administered	
H1 antihistamine	22 (57.9)
H2 antihistamine	10 (26.3)
Intavenous fluids	27 (71.1)
Leukotriene antagonist	1 (2.6)
Albuterol	1 (2.6)
Coricosteroids	16 (42.1)
Acetaminophen	14 (36.8)
NSAID ¹	8 (21.1)
Anxiolytic	8 (21.1)
Meperidine	27 (71.1)
Final Infusion Rate Achieved	
As planned	32 (84.2)
Below planned rate	4 (10.5)
Above planned rate	2 (5.3)
Completed Desensitization	
Yes-discharged home	34 (89.5)
Yes-but sent to ED for evaluation at completion	3 (7.9)
No-terminated early	1 (2.6)

21 ¹Nonsteroidal anti-inflammatory drug ²Emergency Department

22 **Table 4-Comparison of changes to vital sign parameters from baseline to reaction during**
 23 **desensitization**

	Baseline	Peak During Reaction	Mean difference	P value
Temperature (° Celsius)	36.8±0.24	37.4±0.83	0.62±0.80	<0.0001
Heart Rate (beats/min)	85.1±14.8	103.5±15.5	18.4±20.0	<0.0001
Systolic Blood Pressure (mm Hg)	130.8±19.7	156.5±27.5	25.7±29.9	<0.0001
Respiratory Rate (breaths/min)	17.3±1.2	19.8±5.3	2.7±5.7	0.01
	Baseline	Nadir during reaction	Mean difference	
% Oxygen Saturation	98.1±1.6	96.1±2.2	-2.0±1.8	<0.0001

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25 **Table Legend:** Data are reported as means ± standard deviation. A two tailed, paired sample T test was
 26 utilized to determine differences from baseline to peak (or nadir, as indicated) levels achieved during
 27 desensitization. For all, a value of p<0.05 was deemed significant.

28 **Table 5-Correlation between serum IL-6 level during reaction and biophysical changes**

Dependant Variables Tested	Serum IL-6 level during reaction (independent variable)	
	R ²	P value
Peak T	0.019	0.42
Delta T	0.007	0.61
Peak HR	0.034	0.27
Delta HR	0.153	0.015
Peak sBP	2.50 E ⁻⁵	0.98
Delta sBP	0.0008	0.87
Peak RR	0.02	0.39
Delta RR	0.043	0.23
Min SpO2	0.013	0.49
Delta SpO2	0.055	0.163

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30 **Table Legend:** Linear regression analysis evaluating the relationship between serum IL-6 measurements
 31 during reactions (independent variable) and the absolute value or change in vital sign measurements
 32 (dependant variables) as indicated. All associations were weak, with only a weak correlation between IL-6
 33 levels and delta HR meeting statistical significance. Certain reaction events were excluded from absolute value
 34 and delta linear regression calculations on the basis of missing charted values for Temperature, Respiratory
 35 Rate, and SpO2 during reaction in the patients' medical record. T-Temperature; Delta T-change in
 36 temperature from baseline to peak during reaction; HR-Heart rate; Delta HR change in HR from baseline to
 37 peak during reaction; sBP-systolic blood pressure; Delta sBP change in sBP from baseline to peak during
 38 reaction; RR-respiratory rate; Delta RR change in RR from baseline to peak during reaction; SpO2-percent
 39 oxygen saturation; delta SpO2-change in SpO2 from baseline to nadir during reaction.

40 **Table 6-Biomarker changes from baseline to reaction**

	Baseline		Reaction		Mean Difference		P value
	N	Mean±SD	N	mean±SD	N	mean±SD	
IL-6	19	4.7±2.2	Overall (38) Chemo (24) Biologic (13)	627.8±1007.5 669.1±1022.9 599.6±104.8	19	1066.8±1257.7	0.0008
Tryptase	12	8.5±3.0	30	4.6±2.7	9	-0.6±1.6	0.297

41 **IL-6: interleukin 6; SD: standard deviation**

42 **Table Legend:** Not all events had dual measurements of IL-6 and tryptase at baseline and during the
 43 reaction. Total number of reactions for which individual biomarker parameters were obtained are reported
 44 along with their mean and SD at each respective timepoint. For P value mean difference calculation,
 45 calculations were possible for 19 reactions with available baseline and reaction IL-6 levels measured and for 9
 46 reactions with available baseline and reaction tryptases. A one sided, paired T test was employed under the
 47 assumption that IL-6 levels would increase during reaction. A two sided pair T test was employed for serum
 48 tryptase. Statistical significance was predefined as p<0.05. Of the 38 events, elevated IL-6 levels (normal
 49 defined as serum IL-6<17.5 pg/mL) were seen in 29 reactions (76.3% of all reactions). All reactions in which

50 fever was documented ($>38^{\circ}\text{C}$) were associated with elevated IL-6 levels. By comparison, reaction tryptase
51 levels were normal across all events except for one in which a tryptase was obtained and baseline available for
52 comparison (elevated if $>11.5\text{ ng/mL}$ or >1.2 times baseline + 2 ng/mL).

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