

## JNK inhibitors: is there a future?

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### Abstract

JNK is a subfamily of MAP kinases that regulates a range of biological processes implicated in response to stress, such as cytokines, ultraviolet irradiation, heat shock, and osmotic shock as well as growth factors like PDGF, EGF, FGF, *etc.* They were originally identified as kinases that bind and phosphorylate JUN on S63 and Se73 within its transcriptional activation domain. The deregulation of these kinases is shown to be involved in human diseases, such as cancer, immune diseases and neurodegenerative disorders. The realization of the therapeutic potential of the inhibition of JNKs led to a thorough search for small-molecule inhibitors first for research purposes, but later also for therapeutic applications. Here, we discuss some of the most well-known JNK inhibitors and their use in basic research or clinical science.

### Introduction

Protein kinases are a large family of enzymes, which catalyze protein phosphorylation. The phosphorylation of proteins results in a change in localization of protein and/or their function such as interaction with other proteins, affinity or enzymatic activity. The human genome contains more than 510 protein kinase genes. Protein phosphorylation plays a crucial role in the regulation of many cellular functions such as proliferation, differentiation, migration and apoptosis. Because of that, deregulation of kinase activity can result in outstanding alterations in these processes. For example, deregulated kinases are frequently found to be oncogenic and can be essential for the survival of cancer cells.<sup>1</sup> Moreover, the phosphorylation of some proteins, such as ErbB2,<sup>2-4</sup> EGFR,<sup>3,5</sup> Erk,<sup>6-8</sup> SchA,<sup>9</sup> Akt<sup>10,11</sup> and many more, is associated with prognosis in a number of human cancers.

c-Jun N-terminal kinases (JNKs) are members of subfamily of MAP kinase family of serine/threonine kinases which are found in both

unicellular organisms such as yeast and multicellular organisms such as plants, fungi, and vertebrates. There are three different alternatively spliced genes MAPK8 (JNK1), MAPK9 (JNK2), and MAPK10 (JNK3) that produce ten different isoforms. JNK1 and JNK2 are ubiquitously expressed but JNK3 is expressed primarily in the nervous system.<sup>12-15</sup> JNKs are activated by phosphorylation in the activation loop at residues Thr183/Tyr185 (JNK1, JNK2) or pThr-221 and pTyr-223 (JNK3) by the MAP2Ks: MAP2K4 (MKK4), MAP2K5 (MKK5) and MAP2K7 (MKK7), and are dephosphorylated and thus deactivated by MAP kinase phosphatases including DUSP1 (MKP1) and DUSP10 (MKP5). Signaling through the JNK-pathway is organized through binding to scaffold proteins such as MAPK8IP1 (JIP1),<sup>16</sup> MAPK8IP2 (JIP2), MAPK8IP3 (JIP3), SPAG9 (JIP4)<sup>17</sup> or WDR62,<sup>18</sup> which assemble signaling complexes containing MAP3K, MAP2K and MAPKs in addition to JNK-phosphorylated transcription factors such as JUN, ATF2 and ELK1.

JNKs play a central role in the inflammatory signaling system, and thus it is not unexpected that deactivation of JNK signaling is very common in a number of diseases, such as cancers, inflammatory, autoimmune and neurodegenerative diseases. There's plenty evidence showing that JNKs could be considered as therapeutic targets in Parkinson's and Alzheimer's disease,<sup>19,20</sup> obesity and insulin resistance,<sup>21,22</sup> rheumatoid arthritis,<sup>23</sup> asthma,<sup>24-26</sup> vascular disease and atherosclerosis.<sup>27</sup>

The importance of JNKs in many biological processes as well as pathologies led to a serious pursuit for small-molecule inhibitors, which resulted in various small molecules such as aminopyrazoles, aminopyridines, aminopyrimidines, indazoles, pyridine carboxamides, benzothien-2-ylamides and benzothiazol-2-yl acetonitriles to be reported as JNK inhibitors.<sup>28</sup> Here we will discuss most interesting and successful of those.

### JNK inhibitors

AS601245 is a potent and cell permeable ATP competitive JNK inhibitor. IC<sub>50</sub>: JNK1 = 150nM, JNK2 = 220nM, JNK3 = 70 nM (Figure 1). Displays anti-inflammatory properties as shown in an experimental mouse model of rheumatoid arthritis<sup>29</sup> and has been shown to reduce TNF- plasma levels induced by LPS in mice.<sup>30,31</sup> It has also been shown to provide significant protection against the loss of hippocampal CA1 neurons in a gerbil model of transient global ischemia, suggesting that it may be a pertinent approach in the therapy of ischemic insults,<sup>32</sup> it also reduced damage to

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neurites and decreased astrogliosis in a similar study.<sup>33</sup> Likewise, this inhibitor rescued neuronal apoptosis in the developing rat brain after hypoxia-ischemia.<sup>34</sup> AS601245 also decreased cardiomyocyte apoptosis and infarct size after myocardial ischemia and reperfusion in rat model of myocardial ischemia/reperfusion.<sup>35</sup>

Interesting findings have been obtained using AS601245 as an antiviral agent. AS601245 as well as SP600125 inhibited cellular entry and replication of hepatitis C virus.<sup>36</sup> In addition, the application of SP600125 and AS601245 reduced influenza A virus (H7N7 and H1N1v) amplification by suppressing viral protein and RNA synthesis.<sup>37</sup> AS601245 was also found to be a potent inhibitor of HIV-1 reactivation in latently infected primary T cells and T cell lines.<sup>38</sup>

Probably the most interesting are the findings of AS601245 as an anticancer agent. AS601245 decreased cell adhesion and migration via decrease in the fibrinogen release in human colon cancer cells.<sup>39</sup> It has also affected the proliferation of colon cancer cell lines.<sup>40</sup> AS601245 also led T-cell acute lymphoblastic leukemia cells to cell cycle arrest and apoptosis and increased sensitivity to Fas-mediated apoptosis<sup>41</sup> and sensitized promonocytic leukemia cells to arsenic trioxide-induced apoptosis.<sup>42</sup>

AS602801 (Bentamapimod) is a selective JNK inhibitor that has been found to block T-cell proliferation and induce apoptosis. IC<sub>50</sub>: JNK1 = 80 nM, JNK2 = 90 nM, JNK3 = 230 nM (Figure 1). This inhibitor blocked T-lymphocyte proliferation and induced apoptosis in relapsing-remitting multiple sclerosis patients.<sup>43</sup> In addition, it has been shown to induce the regression of endometriotic lesions in human endometrial organ cultures, nude

mice xenograft as well as rat disease models.<sup>44</sup> AEG 3482 is a JNK inhibitor with an  $IC_{50} = 20 \mu\text{M}$  (Figure 1). The action is not direct, since it binds Hsp90 and facilitates HSF1 release, induces expression of Hsp70, which in turn blocks JNK activation. It also reduces apoptosis of neonatal sympathetic neurons after NGF withdrawal.<sup>45</sup> It was also shown to decrease neuron specific toxicity of oligomeric amyloid  $\beta$ .<sup>46</sup>

BI 78D3 is a JNK inhibitor with an  $IC_{50} = 280 \text{ nM}$ . It displays >100 fold selectivity over p38 kinases and no activity towards mTOR and PI-3K (Figure 1). BI 78D3 acts via inhibition of JIP1-JNK binding. It has also been shown to restore insulin sensitivity in mouse disease models of type 2 diabetes.<sup>47</sup> BI-78D3 as well as SP600125 reduced phenylephrine- and norepinephrine-induced contractions of human prostate smooth muscle.<sup>48</sup> Interestingly, BI-78D3 pretreatment sensitized osteosarcoma cells (but not normal osteoblasts) to doxorubicin-induced apoptosis,<sup>49</sup> which gives some ground for thoughts regarding the future combination treatment.

CC-401 is a specific inhibitor of JNK with  $IC_{50} = 25\text{-}50 \text{ nM}$ . It is a second generation ATP-competitive inhibitor selective against JNK over other kinases such as p38, ERK, IKK2 and ZAP70 (Figure 2). It was shown to significantly inhibit renal fibrosis and tubular cell apoptosis, thus suggesting JNK pathway as a potential therapeutic target in progressive kidney disease.<sup>50</sup> In addition, CC-401 blocked reduced proteinuria in rat experimental anti-GBM glomerulonephritis<sup>51</sup> and crescent formation, in a rat model of severe crescentic anti-GBM glomerulonephritis.<sup>52</sup> CC-401 was also shown to decrease hepatic necrosis and apoptosis after orthotopic liver transplantation in rats<sup>53</sup> and hepatic ischemia reperfusion injury.<sup>54,55</sup> CC-401 in combination with oxaliplatin showed synergism in colon cancer cell lines HT29 and SW620 both *in vitro* and in mouse xenografts.<sup>56</sup> CC-401 as well as SP600125 efficiently inhibited human cytomegalovirus replication in cultured human fibroblast.<sup>57</sup>

SP600125 is a potent, selective and reversible inhibitor of JNK enzymes, with  $IC_{50}$ : JNK1, JNK2 = 40 nM JNK3 = 90 nM. Inhibition is over 300-fold more selective for JNK as compared to ERK and p38 MAP kinases (Figure 2). It is the most widely used JNK inhibitor in basic and clinical research, with several hundred papers published. One of the main clinical aspects of SP600125 is the neuroprotection. It was shown to protect dopaminergic neurons from the apoptosis in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of Parkinson's disease<sup>58</sup> protect a transient brain ischemia/reperfusion-induced neuronal death in rat hippocampal CA1 neurons,<sup>59,60</sup> protect cerebellar granule cells against potassium deprivation-induced apoptosis,<sup>61</sup> decrease streptozotocin induced neurocognitive deficit and

oxidative stress in rats,<sup>62</sup> prevent the disruption of blood-brain barrier induced by methamphetamine<sup>63</sup> and diminish neuronal cell death in experimental cerebral malaria in mice.<sup>64</sup> Worth mentioning is anticancer activity of SP600125 as well. It was shown to induce cell death selectively in undifferentiated thyroid cancer cell lines,<sup>65</sup> sensitize the multidrug-resistant KBV20C human oral squamous carcinoma cell line,<sup>66</sup> enhance TGF- $\beta$ -induced apoptosis of human cholangiocarcinoma cell line RBE,<sup>67</sup> suppress glioblastoma cells,<sup>68</sup> selectively kill p53-deficient human colon carcinoma cells in mouse xenograft model,<sup>69</sup> enhance dihydroartemisinin-induced apoptosis in human lung adenocarcinoma cells<sup>70</sup> and reduce the viability of doxorubicin resistant stomach cancer cells.<sup>71</sup> Inflammation is another area where SP600125 has been extensively studied. It was shown to have protective effects in an experimental model of cerulein-induced pancreatitis,<sup>72</sup> diet-induced rat model of non-alcoholic steatohepatitis<sup>73</sup> and mouse model of allergic airway inflammation<sup>74</sup> and promote resolution of allergic airway inflammation in murine acute asthma model.<sup>75</sup> In addition, SP600125 had protective effects of on renal ischemia-reperfusion injury in rats<sup>76,77</sup> and hepatic failure.<sup>78</sup> It also had antiviral activities, as mentioned above as well as suppressed allograft rejection.<sup>79</sup>

SU 3327 is a selective inhibitor of JNK with  $IC_{50} = 0.7 \mu\text{M}$ . As BI 78D3, SU 3327 also acts via inhibition of JIP1-JNK binding ( $IC_{50} = 239$

nM) (Figure 2).<sup>80</sup> Pre-treatment of human astrocytes with either SP600125 or SU 3327, and trauma-induced human astrocyte retraction in *in vitro* study.<sup>81</sup> Inhibition of JNK by SU3327 was shown to aggravate the recovery of rat hearts after global ischemia.<sup>82</sup> SU 3327 was also shown to reduce mitochondrial dysfunction and liver damage in acute liver injury mouse model.<sup>83</sup>

## Tanzisertib (CC-930) and D-JNKI-1 (XG-102, AM-111) - First JNK inhibitors in clinical trials

Tanzisertib (CC-930) is a potent, selective, and orally active JNK inhibitor, developed by Cellgene company, with  $IC_{50}$ : JNK1 = 0.06  $\mu\text{M}$ , JNK2 = 0.007  $\mu\text{M}$  and JNK3 = 0.006  $\mu\text{M}$  and selective against MAP kinases ERK1 and p38 with  $IC_{50}$  of 0.48 and 3.4  $\mu\text{M}$  respectively.<sup>84</sup> Of a panel of 240 kinases, EGFR was the only non-MAP kinase showing  $IC_{50}$  of 0.38  $\mu\text{M}$  (Figure 2). CC-390 was also evaluated in several animal models. In acute rat LPS-induced inflammation model inhibited the production of TNF by 23% and 77% at 10 and 30 mg/kg oral dose respectively. In a mouse bleomycin-induced pulmonary fibrosis model it reduced Lung fibrosis scores by 18-32% in dose dependent manner (25-150 mg/kg CC-930 prior to admin-

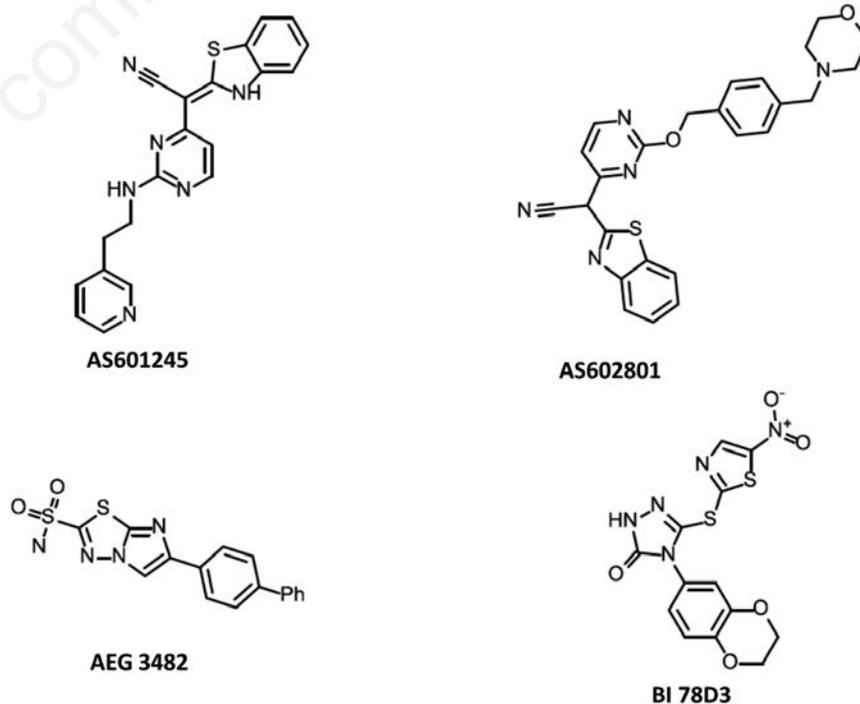


Figure 1. AS601245, AS602801 (Bentamapimod), AEG 3482 and BI 78D3.

istration of bleomycin).<sup>85</sup> Based on animal models and negative toxicity results, CC-930 was selected as a further development candidate for Phase I clinical trial. Results from Phase I trials in healthy male volunteers have indicated that CC-930 was well-tolerated and exposure was dose-proportional, therefore CC-930 was advanced to Phase II clinical trial to characterize the safety, pharmacokinetics, and biological activity in patients with idiopathic pulmonary fibrosis.<sup>86</sup> Trial design was following: 4-week placebo-controlled double-blind treatment phase; CC-930 sequential escalation oral doses: 50 mg QD, 100 mg QD, 100 mg BID; 52-week open-label treatment extension plus 52-week follow-up phase. Preliminary results showed that the change in MMP-7 plasma levels significantly correlated with the change in lung function and there was a decrease in MMP-7 plasma level with increasing CC-930 dose and drug exposure. However, Tazisertib clinical development has been discontinued due to unfavorable risk/benefit profile.

*D-JNKI-1* (XG-102, AM-111) is one of the best known and most used peptide inhibitors of JNK [others being TI-JIP, TAT-TLJIP and L-JNKI (XG-101)]. It is an inhibitory peptide derived from JIP and is blocking JNK-JIP interaction. D-JNKI-1 is the D amino acid-containing retroinverso peptide, derived from the JIP JNK-binding domain sequence<sup>87</sup> (Figure 3). A number of preclinical studies using this peptide inhibitor, resulted in clinical evaluations. In a model of hearing loss using organ cultures of neonatal mouse cochlea exposed to an aminoglycoside and cochlea of adult guinea pigs that were exposed to either an aminoglycoside or acoustic trauma, D-JNKI-1 protected against auditory hair cell death and resulting hearing loss.<sup>88</sup> In addition, delayed phase of hearing loss caused by cochlear implant electrode insertion in guinea pigs can be prevented by D-JNKI-1 treatment of cochlea.<sup>89</sup> In chinchilla model for permanent hearing loss from impulse noise trauma, this inhibitor also had a protective effect.<sup>90</sup> D-JNKI-1 also reduced hearing loss in a guinea pig model of acute labyrinthitis.<sup>91</sup> Based on these preclinical findings, a prospective randomized phase III study was initiated for the intratympanic treatment of acute acoustic trauma with D-JNKI-1.<sup>92</sup> The signs of a therapeutic effect were seen in this study. Some adverse events were reported in 45% (5/11) of study participants; however none of them were serious or severe. Another double-blind, randomized, placebo-controlled phase II study for treatment of acute sensorineural hearing loss was initiated by Auris Medical.<sup>93</sup> Single-dose intratympanic injection of D-JNKI-1 (0.4 or 2.0 mg/mL) or placebo was used in 210 patients within 48 h after acute acoustic trauma or idiopathic sudden sensorineural hearing loss. 0.4 mg/mL showed statistically significant, clinically relevant, and

persistent improvements in hearing compared with placebo; the drug was well tolerated. The same company is currently organizing two crucial phase III clinical trials in the treatment of idiopathic sudden sensorineural hearing loss: HEALOS (Europe/Asia, start Q4/2015) and ASSENT (USA, start Q2/2016). In both trials a single dose of D-JNKI-1 0.4 mg/mL or 0.8 mg/mL will be compared to placebo in patients

suffering from acute severe to profound hearing loss within 72 hours from idiopathic sudden sensorineural hearing loss onset.<sup>94</sup> In addition, a phase II trial in the treatment of surgery-induced hearing loss called REACH (USA; start Q3/2016) is being prepared.<sup>94</sup> D-JNKI-1 will be administered intraoperatively in patients with residual hearing who are undergoing cochlear implant surgery and who are at

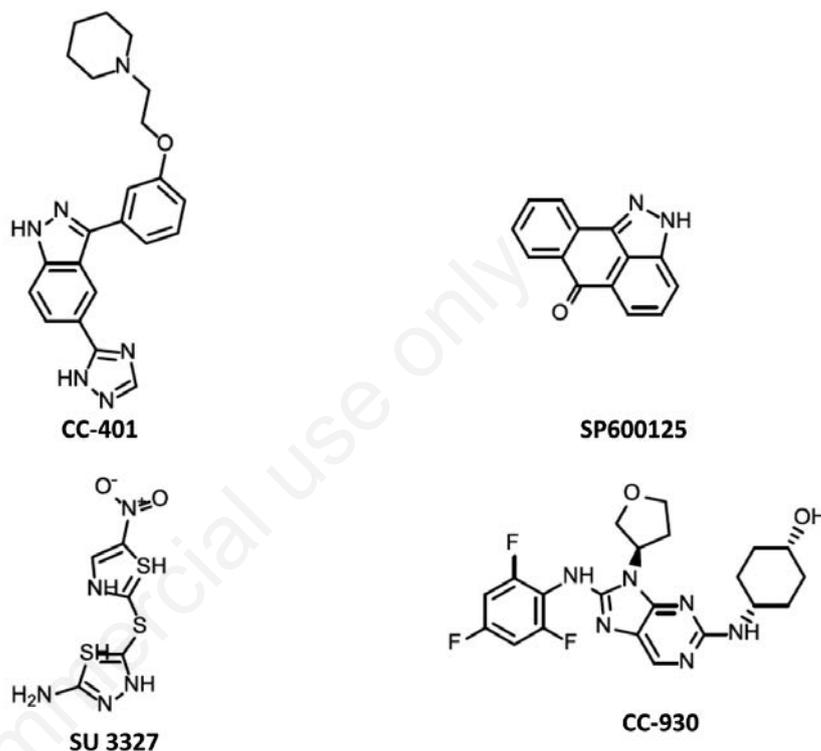


Figure 2. CC-401, SP600125, SU 3327 and CC-930 (Tanzisertib).

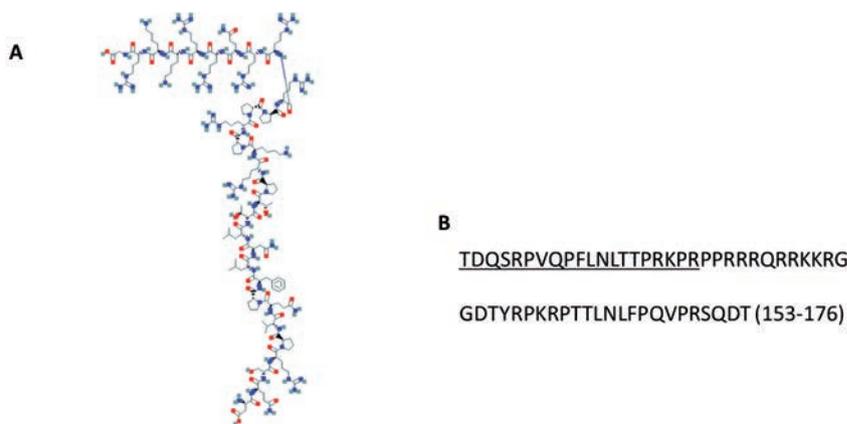


Figure 3. D-JNKI-1 (XG-102, AM-111). A) 2D structure of D-JNKI-1 peptide (copied from <https://pubchem.ncbi.nlm.nih.gov/compound/72941992>). B) D-JNKI-1 peptide amino acids: above - D amino acid-containing retroinverso peptide, with underline amino acids, corresponding to JIP1 protein; under - the JIP1 JNK-binding domain sequence from which amino acids were derived.

risk of losing residual hearing.

Similarly, the preclinical model, such as endotoxin-induced uveitis in rats,<sup>95</sup> led to the indication of the phase I clinical trial in 20 patients with intraocular inflammation.<sup>96</sup> Patients were assigned to 1 of the 4 dose escalating (45, 90, 450, or 900 µg D-JNK1-1) groups of 5 patients each. Drug safe and well tolerated [17 non-serious adverse events, considered unrelated to the study treatment, were reported for 50% (10/10 patients); adverse event incidence was not related to the drug dose], however further studies are required to evaluate its efficacy. Several other pathological conditions have been tested in preclinical animal models, such as cerebral ischemia,<sup>97-100</sup> neuropathic pain,<sup>101</sup> myocardial ischemia-reperfusion injury,<sup>102</sup> hepatic damage,<sup>103,104</sup> middle cerebral artery occlusion,<sup>105</sup> skin cancer,<sup>99</sup> Alzheimer's disease,<sup>106,107</sup> non-alcoholic steatohepatitis,<sup>108</sup> colitis,<sup>109</sup> spinal cord injury<sup>110</sup> and others, suggesting, that many more clinical trials are underway.

## Conclusions and Future Perspectives

Regardless of significant developments in latest years in the development of JNK, many questions are yet unanswered. One of the main concerns is the JNK inhibitor specificity and suitable ways to control it. Another important question is whether the inhibitors selective for individual JNK isoforms are designable. On the other hand, knock-out and/or siRNA studies should help to establish whether isoform-specific inhibitors are at all desirable. So far, judging by phenotypes of JNK1, JNK2 and JNK3 knock-out mice, JNK isoform-selective inhibitors seem to be valuable,<sup>111</sup> however experiences with other kinases show, that in many cases inhibitors with broader selectivity sometimes are more beneficial than very specific ones. The sequence similarity between isoforms could also make it extremely difficult to achieve the specificity. Peptide inhibitors might be able to solve these problems. On the other hand, new approaches, such like computer-assisted, 3D structure based approaches to generate new generations of kinase inhibitors<sup>112</sup> might solve a lot of problems as well. On the other hand, from the studies on other kinase, such as CDKs,<sup>113</sup> it seems that combination therapies are more hopeful than immunotherapies, therefore chemotherapeutic or other agents should be evaluated in combination with JNK inhibitors. Other targeted drugs such as inhibitors of other kinases as well as other enzymes should also be evaluated in combination with JNK inhibition.

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