



Review

Mismatch repair and response to DNA-damaging
antitumour therapiesM. Bignami^{a,*}, I. Casorelli^a, P. Karran^b^a*Laboratorio di Tossicologia Comparata, Istituto Superiore di Sanita', Viale Regina Elena 299, 00161 Rome, Italy*^b*Cancer Research UK, London Research Institute, Clare Hall Laboratories, South Mimms,
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Abstract

Most antitumour therapies damage tumour cell DNA either directly or indirectly. DNA damage responses, and particularly DNA repair, influence the outcome of therapy. Because DNA repair normally excises lethal DNA lesions, it is intuitive that efficient repair will contribute to intrinsic drug resistance. Indeed, in certain circumstances reduced levels of DNA nucleotide excision repair are associated with a good therapeutic outlook (Curr Biol 9 (1999) 273). A paradoxical relationship between DNA mismatch repair (MMR) and drug sensitivity has been revealed by model studies in cell lines. This suggests that connections between MMR and tumour therapy might be more complex. Here, we briefly review how MMR deficiency can affect drug resistance and the extent to which loss of MMR is a prognostic factor in certain cancer therapies. We also consider how the inverse relationship between MMR activity and drug resistance might influence the development of treatment-related malignancies which are increasingly linked to MMR defects.

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1. Mismatch repair and human cancer

DNA mismatch repair (MMR) is a major contributor to the correction of replication errors (for recent reviews, see Refs. [1–4]). The most important proteins are conserved from bacteria to man. In the first step of the repair process in humans, DNA mismatches are recognised by protein heterodimers containing hMSH2. In hMutS α , the most abundant complex, hMSH2 is partnered by hMSH6. The quantitatively more minor hMutS β recognition factor comprises hMSH2 and hMSH3. In a yeast model, MutS α forms a stable ternary complex with proliferating cell nuclear antigen (PCNA) and homoduplex DNA. This interaction provides a plausible general mechanism through which MMR recognition factors might be targeted to replicating DNA. Recognition and binding of

MutS α to base:base mismatches disrupts its binding to PCNA [5]. In addition to base:base mispairs, hMutS α can bind to small loops of one or two bases (called insertion/deletion loops (IDLs)) that are thought to arise through misalignment between the replicating template and daughter DNA strands. hMutS β has a preference for binding IDLs formed by larger loops of two or more bases. The subsequent step of repair involves hMutL α , an hMLH1:hPMS2 dimer that interacts with hMutS α and somehow directs the removal and replacement of a long stretch of DNA containing the mismatch or IDL. These later events involve DNA helicases, nucleases (for example hEXO1) [6] and a DNA polymerase, most likely DNA polymerase δ or ϵ . hMLH1 also forms heterodimers with hPMS1 to form the hMutL β complex, and with hMLH3. The function of hMutL β in MMR is unclear [7]. In rodents, mMLH3 (presumably in concert with mMLH1), plays an important role in meiosis [8].

Hereditary Non Polyposis Colon Cancer (HNPCC) is a familial condition characterised by a predisposition to

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cancers of the colon, endometrium, stomach, ovary, urinary and biliary tracts. In most cases, either *hMSH2* or *hMLH1* is mutated in the germline of HNPCC individuals (for review see Ref. [9]). Mutations in *hMSH6*, *hPMS2* and *hEXO1* are less frequent and are sometimes associated with 'atypical' HNPCC tumour phenotypes. In HNPCC tumours, the second MMR allele is inactivated somatically prior to, or during, tumour development and the tumour is MMR-defective. Inactivation of MMR is associated with the persistence of replication errors and a large increase in spontaneous mutation rate. This mutator phenotype affects point mutations, but its effects are particularly apparent in simple (one- or two-base) repeated sequences which are particularly prone to IDL formation. These may be located in expressed genes, but are more frequently found in non-expressed microsatellite DNA regions. Thus, microsatellite instability (MSI)—numerous alterations in the lengths of microsatellites—is a marker of defective MMR. MSI is used to identify human cancers with MMR defects. Surveys of the frequency of MSI among various tumour types identified MMR defects in 10–20% of sporadic colorectal tumours. Other types of cancer (for example, endometrium, ovary, stomach—and others that are represented in familial forms in HNPCC) are associated with an increased incidence of MSI, suggesting that somatic MMR inactivation is frequent in the development of some cancers [10]. In MSI-sporadic tumours, MMR is generally inactivated by epigenetic gene silencing via methylation of the *hMLH1* gene promoter rather than by mutation [11].

2. MMR and drug sensitivity

Studies *in vitro* and *in vivo* concur that MMR controls the response to the cytotoxic effects of some DNA damaging drugs (Fig. 1). MMR is one of the main determinants of the toxicity of chemicals that produce O⁶-methylguanine in DNA. Thus, tolerance to agents such as *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) and *N*-methyl-*N*-nitrosourea (MNU) is a common characteristic of MMR-defective cells and tumours of human and murine origin [12]. We have suggested that MMR mediates killing by these carcinogens through the recognition and processing of DNA mismatches containing the methylated base, although the contribution of other mechanisms has not been ruled out [13]. It seems likely that the intervention of MMR results in the production of DNA strand-breaks and that this is an essential step towards eventual cell death. DNA strand-breaks activate the signalling pathways that control the cell's apoptotic response (activation of ataxia telangiectasia mutated (ATM), phosphorylation of p53, CHK1 and CHK2, induction of p73, etc.). It has been

shown that many of these events require functional MMR in both cultured cells and in animal models treated with methylating drugs [14–18]. The implications of these modifications for cell death after DNA methylation damage remain undefined. Nevertheless, the recapitulation of methylation tolerance in model human tumour systems such as xenografts suggests that defective MMR is likely to be an important factor in the clinical unresponsiveness of tumours to methylating agent chemotherapy.

3. MMR and therapeutic response

Is there any evidence that loss of MMR affects the clinical sensitivity of tumours to chemotherapy? This is too large a question to answer comprehensively in a brief review. Instead, we have chosen to review the response of MMR-defective cells to chemotherapeutic agents *in vitro* and to investigate possible correlations with the survival of patients affected by cancer of a single site. We focus on colorectal cancer because the prevalence of MSI in both the familial and sporadic forms provides an analytical power that is missing for other tumour types.

5-Fluorouracil (5-FU) and its deoxynucleoside, 5-fluorodeoxyuridine (5-FdUrd), are the most commonly used drugs for colorectal cancer therapy and this clinical choice is reflected by the generally higher sensitivity of colorectal tumour cell lines to 5-FU and 5-FdUrd [19]. The mechanism underlying the therapeutic effect of 5-FU is not fully defined. Cytotoxicity has been linked to 5-fluorouridine monophosphate (5-FUMP) incorporation into RNA and to inhibition of DNA synthesis—either directly following 5 - fluoro - 2' - deoxyuridine - 5'-monophosphate (FdUMP) incorporation, or via FdUMP-mediated inhibition of thymidylate synthase and depletion of the deoxythymidine triphosphate (dTTP) pool. In cultured cells, 5-FU sensitivity is significantly influenced by p53 status. Among the National Cancer Institute (NCI) panel of tumour cell lines, those with p53 mutations are more resistant to 5-FU than those with wt-p53 [19,20]. This differential sensitivity is also apparent in xenografts [21]. Investigations of the responses of MMR-proficient and -deficient cell lines have not produced any overall consensus view of the contribution of MMR to fluoropyrimidine sensitivity. Increased resistance to 5-FU and 5-FdUrd has been reported in the MMR-defective HCT116 (*hMLH1*[−]), LoVo (*hMSH2*[−]) and DLD1 (*hMSH6*[−]) cell lines [22–24]. However, the findings of Aebi and coworkers [25] suggest that MMR status is not a significant factor in fluoropyrimidine sensitivity. In agreement with this, the group of MMR-deficient colorectal carcinoma cell lines in the NCI panel are not obviously distinguished from their repair-proficient counterparts on the basis of 5-FdUrd sensitivity [19]. It is

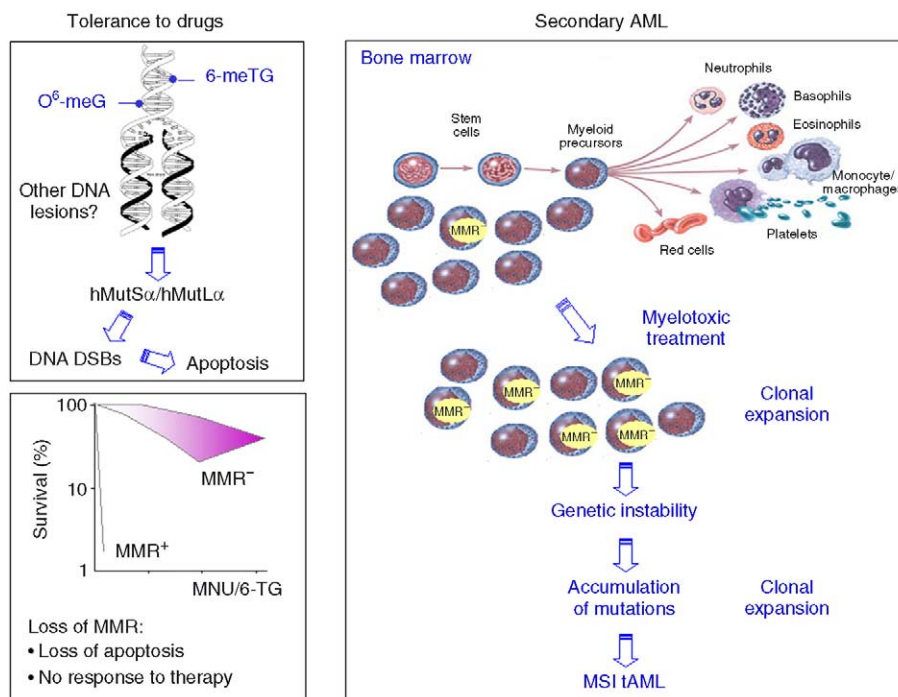


Fig. 1. Left panel: The involvement of DNA mismatch repair (MMR) in DNA lesion-induced cell death. Treatment of cells with methylating agents produces DNA O^6 -methylguanine (O^6 -meG). Similarly, during growth in 6-thioguanine (6-TG), the thiopurine is extensively incorporated into DNA and undergoes a rare non-enzymatic methylation to produce a small number of S^6 -thiomethylguanines (6-meTG). The methylated bases code ambiguously during replication to generate structures that resemble mismatches and that are processed by MMR. This processing is incomplete, and leads to the generation of DNA double-strand breaks (DSBs) that have a high probability of inducing apoptosis. Inactivation of MMR prevents damage processing and severs the connection between DNA damage and apoptosis induction. In the clinic, this would lead to treatment failure. It has been suggested that the resistance to other drugs, such as cisplatin and 5-fluorouracil (5-FU), also reflects the loss of MMR-mediated communication between DNA damage and apoptotic pathways. Right panel: Development of microsatellite unstable acute myeloid leukaemia (MSI AML) in patients treated with DNA damaging drugs. Rare MMR-defective myeloid precursor cells have a selective survival advantage when cancer patients or organ transplant recipients are treated with drugs for which myelotoxicity is dose-limiting. This permits their clonal expansion. The mutator phenotype and hypermutability by DNA damaging drugs that is characteristic of MMR-deficient cells accelerates the development of secondary myelodysplastic syndrome and AML that retains the MSI phenotype.

clear that the other factors that play a significant role in fluoropyrimidine sensitivity may override any effect of MMR. This may underlie the observation that, although *mlh1*-defective mouse fibroblasts are apparently somewhat more resistant to 5-FU or 5-FdUrd than their *mlh1*-corrected counterparts [24], sensitivity to 5-FU is not changed by inactivation of *pms2* if the fibroblasts are p53-defective [26].

Descriptive studies generally suggest that MSI is associated with a better prognosis, at least for hereditary colorectal cancers (reviewed in Ref. [27]), although the reasons for this are unclear. Table 1 summarises representative studies on MSI as a prognostic marker. The impression of a generally more favourable outcome is supported by studies involving Finnish, Danish and Japanese HNPCC patients [28–30], although these contrast with the absence of a substantial survival advantage among Italian HNPCC patients [31,32]. This latter finding for familial Italian colorectal cancer cases might reflect the genetics of the population and/or the impact of screening programmes for early cancer detection. MSI was also found not to be a favourable prognostic

marker in very early onset (<30 years) disease which was associated with germline *hMSH2* or *hMLH1* mutations [33]. In the same study, MSI was a positive prognostic indicator in tumours in an older (>60 years) cohort of patients. Thus, although in many cases MSI is associated with better patient survival, exceptions exist, even among familial cancers.

In sporadic cancers, MSI is generally a good prognostic marker [34–41], although again, exceptions have been reported [42–44]. Are MSI tumours less aggressive and does this, rather than their response to adjuvant therapy, underlie a better prognosis? MSI colorectal carcinomas constitute a distinct subset of tumours which, in addition to their MMR defect, are associated with distinctive clinical and pathological features [45]. These include: early onset, right side location, poor differentiation, extracellular mucin production, peritumoral lymphoid reaction and intratumoral lymphocytic infiltration, diploidy and a lower probability of p53 overexpression. Several studies have addressed the extent to which the better outcome is the consequence of an increased sensitivity to 5-FU-based adjuvant therapy.

Table 1
MSI and survival in colorectal cancer patients

Authors	Year	Total cases	HNPCC/MSI	Sporadic/stable cases	Stage	End point ^a
Lothe ^{b,*}	1993	238	21 MSI	217	A–B–C–D	6.5-y survival
Bubb*	1996	215	23 MSI	196	A–B–C–D	3-y survival
Sankila*	1996	14 261	175 HNPCC	14 086	A–B–C–D	5-y survival
Fujita*	1996	1749	145 HNPCC	1604	A–B–C–D	5-y survival
Myrhoj ^{b,*}	1997	978	108 HNPCC	870	A–B–C–D	5-y survival
Percesepe ^c	1997	462	85 HNPCC	377	I–II–III–IV (TNM)	5-y survival
Bertario	1999	2179	144 HNPCC	2035	A–B–C–D	5-y survival
Halling ^{b,*}	1999	508	76 MSI-H	335	B2–C	8-y survival RFS
Salahshor	1999	181	22 MSI	159	A–B–C–D	5–10-y survival
Feeley	1999	50	5 MSI-H	40	A–B–C	5-y survival
Gryfe*	2000	607	102 MSI	485	I–II–III–IV (AJCC)	5-y survival
Hemminki ^{d,*}	2000	95	11 MSI	84	C	3-y survival RFS
Elsaleh ^{c,*}	2001	891	21 5-FU/ levamisole	241	III (AJCC)	6.5-y survival
			42 surgery	428	III (AJCC)	
Samowitz*	2001	1026	184	n.s.	I–II–III–IV (AJCC)	5-y survival
Guidoboni*	2001	245	47 MSI-H	62	II–III (TNM)	5-y survival RFS
Liang ^{c,*}	2002	169 5-FU/leucovorin	35 MSI	134	IV (AJCC)	RR-MST
		75 surgery	17 MSI	58	IV	
Barratt	2002	393	89 MSI	279	B–C	5-y survival
Farrington ^{f,*}	2002	292	25 (<30 y) 12 (>30 y)	28 (<30 y) 162 (>30 y)	A–B–C–D	5-y survival

A–B–C–D, Dukes' classification; AJCC, American Joint Committee on Cancer; RFS, recurrence-free survival; n.s., not specified; RR-MST, response rate-median survival time; MSI, microsatellite unstable; 5-FU, 5-fluorouracil; y, years; HNPCC, hereditary non-polyposis colon cancer; MSI-H, MSI-high.

^a Studies included death from all causes.

^b Correction for staging reduces the *P* value to a non significant level.

^c Significant for Dukes' stage C only

^d Significant for RFS

^e Significant for patients given adjuvant treatment.

^f Significant for patients aged > 30 years.

* Significantly better survival for MSI patients.

The prevailing evidence indicates that MSI is indeed a favourable indicator for response to chemotherapy [38,46,47]. However, no survival benefit from adjuvant chemotherapy was observed among patients with mutated or overexpressed *p53* [47,48]. In view of the strong inverse correlation between MSI and *p53* mutations, these observations suggest that, although MSI is associated with a better therapeutic response, there is not necessarily a direct mechanistic connection between MMR deficiency and improved patient survival.

Is the mutator phenotype itself disadvantageous for the tumour and is this seen as an intrinsically lower aggressiveness of MSI tumours? MSI tumours are generally associated with peritumoral lymphoid reaction and intratumoral lymphocytic infiltration [45]. Lymphocytic invasion is considered to be indicative of a local anti-tumour response. This may be particularly significant in MMR-defective tumour cells which continuously produce abnormal peptides as a consequence of their extreme mutator phenotype [49,50,51]. Thus, part of the improved prognosis of MMR-defective tumours may be due to this localised antitumour immune response [51,52]. In view of the conflicting evidence for the effects of MMR defects on 5-FU resistance in tumour cells *in vitro*, it is interesting to note the suggestion that fluoropyrimidine-based regimes

may enhance the effectiveness of immunotherapeutic treatments [53].

Genes that contain IDL-prone runs of repeated bases in their coding sequences are particularly susceptible to mutational inactivation in MSI tumours [54]. Defective MMR is associated with mutations in *BAX*, *TGFβRII*, *MBD4*, *hMSH3*, *hMSH6*, *RIZ* and *MRE11*. Although a systematic evaluation of the prognostic values of these genetic alterations is available only for *BAX* and *TGFβRII*, they do not seem to have a significant impact on prognosis [55].

4. MMR and acquired drug resistance

There is general agreement that MMR defects are associated with drug resistance in cultured cell lines (Fig. 1). Cultured cells selected for drug resistance can exhibit extreme phenotypes because of multiple changes. The degree of resistance in a tumour may well be less as the tumour cells may have only a limited number of changes affecting sensitivity. It is an article of faith, however, that even small, <2-fold, increases in drug resistance can translate into therapeutic failure because of the development of resistant disease [56]. In the case

of MMR, the difference in sensitivity between repair-proficient and -defective variants is highly dependent on the drug. For example, MMR-defective cells are up to 100-fold more resistant to methylating agents, around 10-fold more resistant to thiopurines, but ≤ 2 -fold more resistant to other agents such as cisplatin [56–58]. In each case, all the increased resistance can be ascribed to inactivation of this single repair pathway. Cultured MSI tumour cells are slightly (≤ 2 -fold) resistant to some platinum-based drugs. Consistent with this, there is a significant enrichment for MMR-deficient cells when mixed cultures of repair-proficient and -deficient cells are exposed to multiple rounds of cisplatin treatment [59]. A similar proliferative advantage when challenged by drugs is observed for repair-defective human tumour xenografts [60–62]. The extent to which these observations in model systems are relevant to human cancers has been examined by measuring MMR protein expression in ovarian tumours before and after cisplatin treatment. A decrease in the extent of expression of MMR proteins—in particular of hMLH1—generally follows several cycles of treatment [61,63,64]. These observations are compatible with the eventual outgrowth of a MMR-deficient drug-resistant tumour. However, this may not always be reflected in a decreased patient response to treatment [64]. MMR deficiency has also been associated with low level resistance to other drugs [65–67]. It has even been suggested that the emergence of MMR-defective disease can be viewed as the development of a type of multidrug resistance [68].

5. MMR and secondary cancer

Antitumour drugs are indiscriminate and do not generally selectively damage tumour cell DNA. There is mounting evidence to implicate drug treatment in the emergence of MMR-deficient cells in tissues other than the site of the primary tumour. The incidence of therapy-related or secondary malignancy—a tumour at a different site to the first tumour that is a direct consequence of the therapy—is increasing. In particular therapy-related AML/MDS (tAML) now accounts for around 10% of all AML [69]. tAML and *de novo* AML are clinically distinct diseases [70] and they respond very differently to therapy. Unlike *de novo* AML, tAML is refractory to treatment and good responses are rare [71]. There is a growing consensus that a significant fraction of tAML cases—probably more than 50%—are MSI [72–75]. MSI is rare in *de novo* AML and is present in $< 5\%$ of cases [73,75,76]. tAML is particularly associated with treatment for a primary Hodgkin's or non-Hodgkin's lymphoma or breast cancer [71] and MSI tAML with drug treatment rather than radiotherapy [75]. The reason why most tAMLs are MSI is not

known. We have suggested MSI AML represents selection of a clone of myeloid cells in which inactive MMR confers a survival advantage for cells challenged by therapeutic drugs (Fig. 1). Two factors are noteworthy in this regard. Firstly, bone marrow cells seem relatively poorly protected by DNA repair that removes potentially lethal DNA lesions—particularly DNA methylation damage [77–79]. Secondly, myelotoxicity is dose-limiting for many chemotherapeutic regimes and drug treatment would provide ideal conditions for the selection of drug-resistant MMR-defective myeloid cells. In cultured cells, inactive MMR is particularly associated with resistance to methylating agents and thiopurines. In the clinic, Hodgkin's and non-Hodgkin's lymphoma are often treated with mixtures of drugs that include a methylating agent such as procarbazine or dacarbazine. The association between a primary lymphoma of this type and tAML is consistent with a role for drug resistance in MSI tAML. However, it has not, so far, been possible to implicate methylating agents directly in the development of MSI tAML. The thiopurines, 6-TG, mercaptopurine, and azathioprine (a thiopurine pro-drug) are all immunosuppressants used in the treatment of autoimmune disorders. Azathioprine, usually combined with Cyclosporin A and steroids, is administered to prevent graft rejection in organ transplant patients. Azathioprine is associated with a significant excess of AML in solid organ transplant is associated with a significant excess of AML and the majority of these therapy-related AMLs are MSI (Offman *et al.* personal communication). Selection for drug resistance related to inactive MMR provides a simple unifying explanation for the excess of MSI AML among cancer chemotherapy patients and organ transplant recipients.

6. New approaches to exploit a repair-deficient phenotype for therapy

With the possible exception of 5-FU and colorectal cancer, the association of MSI with drug resistance and with tAML is generally rather dismal in terms of patient wellbeing. The properties of MMR-defective cells are under continuous investigation *in vitro*. Have these studies defined any properties that might be exploited clinically? Sensitivity to crosslinking agents such as lomustine (CCNU) or mitomycin C [80,81], as well as to the topoisomerase I inhibitors, Camptothecin and Irinotecan (CPT11) [82–84], has been reported in MMR-defective cell lines. There are, however, exceptions to this hypersensitivity. Loss of p53 affects the cytotoxicity of both these types of drugs, but in opposite directions: mutant p53 confers resistance to CCNU [85], but sensitisation to CPT11 [82,86]. In view of the inverse correlation between p53 mutation and MSI, these observations suggest that a judicious choice of drug

treatment might translate into benefit for patients with MSI tumours.

More recently, MMR defects have been linked to a defective S-phase checkpoint after ionising radiation [87,88]. In addition, MMR is required for the correct phosphorylation of the CHK2 protein and an efficient G₂-M cell-cycle arrest after ionising radiation [89,90]. Importantly, neither checkpoint defect is associated with a large difference in sensitivity to ionising radiation (Fig. 2). This raises the interesting possibility that treatments designed to trigger cell-cycle checkpoints might be combined with cell-cycle phase-specific drugs to selectively eradicate MMR tumour cells and spare the surrounding normal cells.

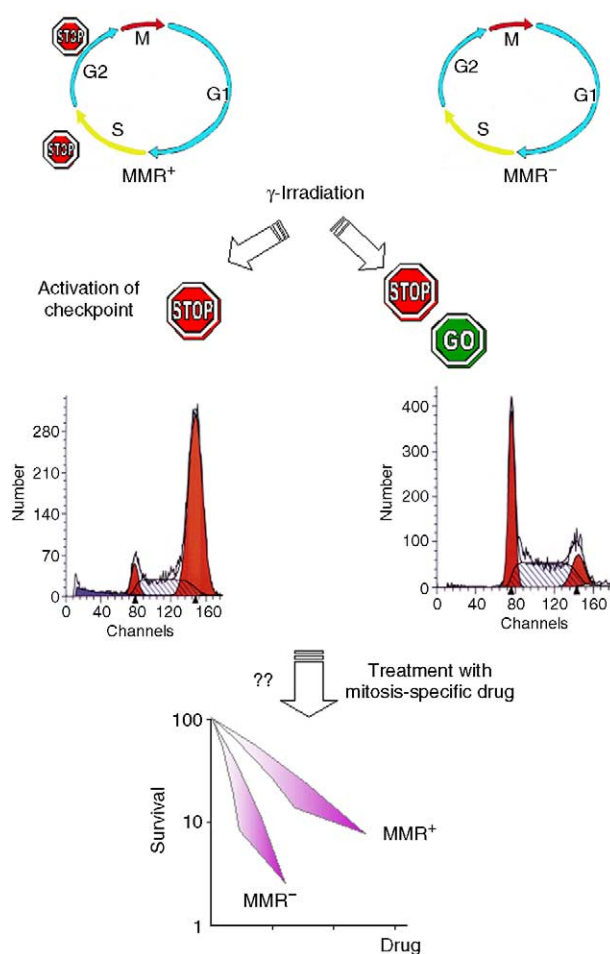


Fig. 2. Possible approaches to selectively eradicate MSI tumour cells by exploiting their cell-cycle checkpoint defects. MMR-deficient cells are defective in cell-cycle checkpoints (STOP). They continue DNA replication and are unable to maintain G₂ arrest after low doses of ionising radiation (1–2 Gy). The figure illustrates how the failure to sustain a G₂ arrest might be exploited. A mixture of MSI tumour and normal cells is treated with a low dose of radiation. Normal cells enter a prolonged G₂ arrest (STOP). The G₂ arrest is only transient in the MSI tumour cells (STOP/GO). During the period that the normal cells remain arrested, the cycling tumour cells should be more susceptible to killing by drugs that target non-G₂ cells. Examples might include mitotic poisons such as vincristine.

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