The Phenotypes Associated with ret Mutations in the Multiple Endocrine Neoplasia Type 2 Syndrome

Bruce A. J. Ponder

Abstract
Different specific mutations in the ret tyrosine kinase give rise to different clinical types of the inherited cancer syndrome multiple endocrine neoplasia type 2 (MEN 2). The explanation for these genotype-phenotype correlations is not yet certain. Several lines of evidence suggest that they result either from different levels of RET activation induced by crine neoplasia type 2 (MEN 2). The explanation for these genotype-phenotype correlations is not yet certain. Several lines of evidence suggest that they result either from different levels of RET activation induced by different mutations or, in one class of mutation, possibly from altered substrate specificity of the RET tyrosine kinase. ret is expressed during development in a lineage of neuroectodermal cells that gives rise to the thyroid C cells and the adrenal medulla, which are involved in tumor formation in MEN 2. ret is also expressed in the enteric autonomic nervous system. Inactivating mutations of ret lead to Hirschsprung’s disease, a congenital absence or maldevelopment of the enteric plexuses, whereas activating mutations in one variety of the MEN 2 syndrome lead to their overgrowth. The range of phenotypic expression seen in families with different ret mutations and the variation within families with the same mutation provide a potentially interesting and tractable system for the analysis of both the relationship between phenotype and genotype and the effects of modifier genes.

Introduction

ret and RET Ligands. The ret gene maps on chromosome 10q11.2. It encodes a transmembrane protein that belongs to the RTK aa superfamily (1). Three isoforms of RET are generated by alternative splicing at the 3' end of the gene: (a) a 1072-aa (short isoform); (b) a 1106-aa protein (intermediate isoform); and (c) a 1114-aa protein (long isoform), respectively (2, 3).

The receptor comprises (Fig. 1): (a) a signal peptide of 28 aa that is cleaved during translation; (b) an extracellular portion that contains a cadherin homology domain (4) and a cysteine-rich region (the cysteines are probably involved in the formation of disulfide bonds, allowing the acquisition of the correct secondary structure); (c) a transmembrane domain; and (d) an intracellular portion that contains the tk domain divided in two subdomains, tk a and tkb, separated by 28 aa. The intracellular portion contains tyrosine residues that undergo autophosphorylation upon ligand activation.

The extracellular portion of the molecule contains several glycosylation sites. Western blot analysis shows two RET isoforms of Mr 150,000 and 170,000 that are glycosylated differently. The Mr 150,000 is the fully mature version of the receptor and is present on the cytoplasmic membrane. The Mr 170,000 isoform is a maturation intermediate that is present only on the endoplasmic reticulum (5).

On the cell membrane, RET is part of a multiprotein complex with glycosylphosphatidylinositol-linked proteins that function as coreceptors (6–9). The binding of the coreceptors increases the affinity of RET for the ligand, the binding of which results in dimerization and activation of the tk function, with consequent autophosphorylation. Three coreceptors have been identified as GFRα1, GFRα2, and GFRα4 (6–13). They increase the receptor affinity for three ligands: (a) GDNF; (b) NTN; and (c) persephin. GDNF and NTN are 42% identical at the protein level, whereas GFRα1 and GFRα2 are 42% identical and 63% similar (14). Persephin has a 40% homology to GDNF and NTN (15). GDNF binds preferentially to the GFRα1/RET complex. NTN, in contrast, binds the GFRα2/RET complex with a higher affinity than the GFRα1/RET complex. Persephin can bind RET only in an interaction with GFRα4. The ability of the ligands and coreceptors to activate RET has been demonstrated by in vitro binding experiments and by their ability to promote ret autophosphorylation and the survival of neuronal cells in vitro (13–16).

Expression. Expression patterns during embryogenesis and after birth suggest a role for RET and for coreceptors GFRα1 and GFRα2 and ligands GDNF and NTN in the development of different central and peripheral nervous system structures and the excretory apparatus. Differences in the expression of the various coreceptors and ligands suggest that they have partially distinct functions. Details of these patterns can be found in several recent publications (17–19) and will not be pursued here.

The Role of ret in the Development of the Tissues Affected in MEN 2

Four tissues are principally affected in MEN 2: (a) the C cells of the thyroid, which secrete the hormone calcitonin; (b) the adrenal medulla; (c) the parathyroid; and (d) the enteric autonomic nerve plexuses (for a review, see Ref. (20); Table 1). The thyroid C cells, enteric nerve plexuses, and adrenal medulla are derived from the neural crest (21). The cells that will give rise to the thyroid C cells come from the vagal neural crest and migrate to the caudal portion of the fourth branchial arch, the site of the ultimobranchial body in birds (Fig. 2). From there, they migrate into the central portion of each thyroid lobe to form the C cells. While in the branchial arch, they are adjacent to the pharyngeal endoderm, from which the parathyroids are derived. During mouse development, both the migrating neural crest cells and the pharyngeal endoderm express RET (22, 23); the expression of RET by this endoderm may account for the involvement of the parathyroids as a component of the MEN 2 syndrome. The adrenal medulla (and other components of the sympathoadrenal chain that are also occasionally involved in MEN 2) originates from the trunk neural crest, whereas the enteric autonomic nervous system is derived from RET-expressing cells that originate in the hindbrain neural crest and migrate with the vagus nerve (24).

In vitro data show that RET acts as a survival factor for the developing enteric neuroblasts (25); thus, the effect of ret loss of function mutations in causing HSCR is easily understood. However, the role of ret signaling in the development of thyroid C cells, the adrenal medulla, and the parathyroids is not clear from the in vitro studies. The mechanism by which ret mutations lead to tumor formation in MEN 2 syndrome is not yet known.

An alternative approach to the analysis of ret function is to create genetically engineered mice bearing either inactivating or activating mutations. ret knockout mice show no obvious phenotype when only
central respiratory failure, variable kidney dysplasia, and a HSCR-like domain, a cytoplasmic tk domain, and three 3' alternative splice forms (see the text).

one allele is lost, but homozygotes die at birth with a variety of abnormalities, including hypoventilation that has been ascribed to central respiratory failure, variable kidney dysplasia, and a HSCR-like absence of gut enteric neuroblasts (26). In contrast, the thyroid C cells and the adrenal medulla appear grossly normal. It has been suggested that the C cells have a dual origin from neuroectodermal and pharyngeal endodermal precursors (Refs. (27) and (28); Fig. 2). If so, the presence of C cells in the ret knockout mice might reflect a ret-independent endodermal lineage, with a ret-dependent neuroectodermal population being absent. However, a simpler explanation is that the C cells are present because ret signaling is not necessary for the growth and survival of the C cells and adrenal medulla, at least until birth. In support of this argument, immunocytochemical studies suggest that RET protein expression is low in embryonic and neonatal mouse thyroid C cells and rises at 4–6 weeks of age (29). An analogy with the role of the receptors of the trk family (30) in the development of sensory neurons suggests that C cells may be dependent on an unknown growth signal before birth but switch to dependency on ret at a later stage. This hypothesis could be tested by constructing mice carrying a conditionally inactive ret gene on an otherwise ret-null background.

In addition to ret knockout mice, transgenic mice have been constructed in which an active MEN 2A-ret gene is expressed from a calcitonin/calcitonin-related gene peptide promoter, giving restricted expression, including in thyroid C cells, from about day E15 (31). Both sets of mice developed multiple foci of hyperplasia of the thyroid C cells, similar to the condition seen in the thyroid and adrenal medulla of human carriers of a MEN 2 mutation (32). However, there is as yet no information about the molecular or developmental phenotype of the hyperplastic cells that would provide a clue to the nature of the changes that these cells have undergone as a result of the ret mutation.

**The MEN 2 Syndrome**

There are three main clinical varieties of MEN 2, which are defined by the pattern of tissues involved (Ref. (8); Table 2). C-cell tumors of the thyroid (MTC) are present in the majority of gene carriers, pheochromocytomas of the adrenal medulla are present in about 50% of gene carriers, and parathyroid hyperplasia or adenoma is present in 10–30% of gene carriers, depending upon how carefully it is sought. The clinical varieties of MEN 2 run true to type and can be ascribed to the different phenotypic effects of the different predisposing mutations discussed in detail below. However, within each type, there can also be considerable variation between members of the same family regarding the presence of one or several of the features of the disease and the age at onset (34). This within-family variation is presumably the result, in unknown proportions, of a combination of chance, environmental influences, and other (modifier) genes. This will also be discussed briefly below.

The different clinical varieties of MEN 2 are associated with different specific point mutations of ret, which result in aa substitutions and activation of the ret tk. The mutations fall into three main groups (8, 35–38): (a) those affecting cysteine residues 609, 611, 618, 620, 630, or 634 in the extracellular domain (additionally, in one family, a mutation has been described affecting non-cysteine codon 631, and in another family, a 12-bp insertion creating an additional cysteine has been described; Refs. (39) and (40); (b) those affecting codons 768, 790, 791, 804, 844, or 891 in the tk domain, which, with one exception to date (38), have been found only in families with FMTC; and (c) those affecting codons 883 or 918 in the tk domain, which are associated exclusively with MEN 2B.

Cysteine mutations have been reported in sporadic tumors but are uncommon (45).

**Mechanisms by Which MEN 2 Mutations Activate the ret tk**

The cysteine mutations activate RET by causing ligand-independent dimerization (46). Nothing is currently known of the three-dimensional structure of the extracellular domain, but presumably, these cysteines normally form intramolecular disulfide bridges, and the substitution of one partner cysteine by another aa as a result of mutation results in an unpaired cysteine, which links with the similar

---

4 Reynolds et al., manuscript in preparation.
The clinical types of MEN 2 are defined by the pattern of tissues involved. Because adrenal and parathyroid involvement is an inconstant feature of MEN 2A, there will be some small families that are in fact MEN 2A but in whom C-cell tumors (MTC) are presumably the only feature. To minimize such a misclassification, we have adopted an arbitrary definition of FMTC as requiring at least four proven cases of MTC in the family, with at least partial evidence from screening of unaffected at-risk family members that adrenal and parathyroid involvement are absent. Families with fewer than four cases of MTC or in whom such evidence is not available were placed into an "unclassified" category (33).

**Table 2. The clinical types of MEN 2 are defined by the pattern of tissues involved**

<table>
<thead>
<tr>
<th>Clinical varieties of MEN 2</th>
<th>MEN 2A</th>
<th>MEN 2B</th>
<th>FMTC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid C cells</td>
<td>Tumor</td>
<td>Tumor</td>
<td>Tumor</td>
</tr>
<tr>
<td>Adrenal medulla</td>
<td>Tumor (~50%)</td>
<td>Tumor (~50%)</td>
<td>Not involved</td>
</tr>
<tr>
<td>Parathyroid</td>
<td>Hyperplasia/benign tumor (10-30%)</td>
<td>Not involved</td>
<td>Not involved</td>
</tr>
<tr>
<td>Enteric ganglia</td>
<td>Normal*</td>
<td>Hyperplasia</td>
<td>Normal</td>
</tr>
<tr>
<td>Other developmental abnormalities</td>
<td>None</td>
<td>Various*</td>
<td>None</td>
</tr>
</tbody>
</table>

* Usually there is no abnormality of the enteric ganglia in MEN 2A, but a few families are described (see text) in whom there is an absence of the ganglia from a variable length of intestine in some individuals.

**Mechanisms of the Genotype/Phenotype Effects**

In principle, one could imagine that activating mutations of ret might have their effects in one or more of the following methods: (a) by activating RET at a different intensity; (b) by activating RET at the wrong time; (c) by activating RET to signal down the wrong pathways; and (d) by activating RET in the wrong place.

It is likely that the first three mechanisms operate in the MEN 2 syndromes, and the extent to which each is active may in part determine the phenotypes that result. The last mechanism, activation in the wrong place, is seen in human disease although not in MEN 2. It occurs in various somatically rearranged forms of RET (PTC-RET) that are expressed in thyroid epithelial cells and give rise to papillary thyroid tumors (57, 58). This category will not be discussed further.

**Activation of RET at Different Intensities.** There is evidence from *in vitro* studies using transfected cells with and without exposure to ligand that different ret mutants differ substantially in the degree to which they activate the tk domain and the degree to which this activation can be further enhanced by binding of the ligand (50, 52, 53, 59, 60). The effects correlate very plausibly with the phenotypes seen in man and suggest that quantitative effects may be the major determinants of the different MEN 2 phenotypes.

**Cysteine Mutations.** Mutations of codons 609, 611, 618, and 620 are most likely to be associated with FMTC and are less frequent in families with MEN 2A syndrome. Transfection studies indicate that in comparison with codon 634 mutations, these mutations are weaker in both cellular and biochemical assays of RET activity (52, 59, 60). They differ from the codon 634 mutants both in their efficiency of dimerization and in their maturation to the fully glycosylated 170,000 form of RET, which is necessary for insertion into the plasma membrane and for signaling. The activity of none of the cysteine mutants is augmented by ligand binding (52), although it should be

cysteine in an adjacent molecule (46). There is a strong correlation between the cysteine codon involved in the mutation and the MEN 2 phenotype (Refs. (33) and (47); Fig. 4). In families with MEN 2A in which pheochromocytoma and parathyroid each occur in at least one family member, almost all of the mutations are in codon 634. In families with MTC and parathyroid disease but without cases of pheochromocytoma or in FMTC, the mutation rate is more frequent in codon 609, 611, 618, or 620. There may be an effect not only of the position of the aa substitution but also of the particular aa substitution involved; however, this remains controversial (33, 48). In 169 families with a codon 634 mutation in the combined study by the RET International Consortium, the C634R mutation was present in 88 families and was by far the most common change, whereas this specific mutation was present in 0 of 9 well-documented FMTC families with a mutation at codon 634 (*P* = 0.007; Ref. (47). Why mutation C634R should be so common is not clear. The mutation has been reported on many different haplotypes (49), suggesting that founder effects are not a major contributing factor, and inspection of the DNA sequence does not suggest an explanation based on the frequency of specific mutational changes. *In vitro* studies have shown no any clear differences in the biochemical properties of different codon 634 mutations (46, 50).

The mutations in the tk domain fall into two groups, as outlined above: (a) those associated primarily with FMTC (codons 768, 790, 791, 804, 844, and 891; Refs. (36), (38), and (51); and (b) two mutations [codons 883 (44) and 918 (41–43)] that are always associated with MEN 2B. All of these tk domain mutations can activate the receptor independent of ligand binding and without dimerization, but in the case of MEN 2B mutations (and probably the other tk domain mutations as well, although this has not yet been reported), the intensity of signaling from the mutant receptor can be further increased by the binding of the ligand (52, 53). An analogy with receptors of similar structure suggests that the MEN 2 mutations induce conformational changes that remove the need for phosphorylation of tyr-905 in the activation loop of the kinase domain before activation can occur (44).

The M918T mutation that is seen in the majority of cases of MEN 2B also has an effect on the substrate specificity of the kinase (54), which may be shared by the more recently described A883F mutation, although this has not been documented. Residue 918 is predicted from protein modeling studies to lie at the base of a pocket in the protein that is involved in substrate binding (42, 55). The substitution of threonine for methionine is predicted to alter the dimensions of the pocket and thus the substrate specificity. Tks fall into two classes: (a) RTKs; and (b) cytoplasmic tks. Almost all RTKs have methionine at the equivalent position to codon 918, whereas almost all cytoplasmic tks have threonine at this position (55). Songyang et al. (54) used degenerate peptide libraries to demonstrate that whereas RTKs prefer hydrophilic aa at positions +1 and +3 downstream of the target tyrosine in their substrate, cytoplasmic tks prefer a hydrophilic residue at +1 and a hydrophobic residue at +3. These different aa contexts flanking the tyrosine provide different preferred substrates for different groups of SH2 domains on signaling molecules and hence the possibility of different pathways of downstream signaling. When wild-type (i.e., MEN 2A) and MEN 2B RET tks were compared for their ability to phosphorylate model tyrosine-containing substrate peptides, a clear shift was seen toward the specificity characteristic of a cytoplasmic tk in the case of MEN 2B. The inference that MEN 2B tk has an altered downstream pathway of signaling is supported by the observation that MEN 2B RET differs from activated wild-type RET in both the pattern of tyrosine phosphorylation of the RET protein itself (46, 56) and in the patterns of tyrosine phosphorylation seen in cell extracts.
The M918T mutation associated with MEN 2B leads to ligand-independent activation of the kinase that can be further enhanced by ligand binding (52, 53). The resulting combined activation is almost certainly stronger than that provided by the Cys-634 mutations (52, 61). Studies of the effects of ligand binding on the more proximal tk domain mutations that are associated with FMTC have not yet been reported.

**Activation of RET at the Wrong Time.** Detailed information about the expression of RET and its ligands (and thus about RET activity during development) is still lacking. It must therefore remain no more than a reasonable conjecture that the hyperplasias of the parathyroid cells may not express the signaling pathways from parathyroid; however, this has yet to be demonstrated. Alternatively, the parathyroid cells may not express the signaling pathways responsible for the particular features of this syndrome and may also be responsible for the frequent involvement of MEN 2B mutations in sporadic tumors. However, the relative contribution of altered specificity and the high level of activation of the receptor provided by the mutation and by additional ligand induced activation is not yet clear. In assessing this, it will be of interest to compare the biochemical effects of the M918T and the A883F mutations, which have an indistinguishable clinical phenotype (44).

**Speculation and Synthesis.** A reasonably clear correlation seems to appear from the above data, which is that the FMTC phenotype is associated with the lowest levels of RET activation in cysteine mutations other than those at Cys-634, presumably, although this has not been published, in the FMTC mutations in the tk domain. Increasingly complete MEN 2A phenotypes are associated with higher levels of activation, and the MEN 2B phenotype with the highest levels of all resulting from the combined effects of the activating mutation and additional ligand stimulation (52, 53). A further explanation for the developmental and other features of MEN 2B may be the altered substrate specificity conferred by those mutations.

An interesting paradox, which can possibly be explained in terms of the quantitative effects of RET signaling on the phenotype, is provided by the families (several have now been described, Refs. (63–67) in which the HSCR (loss of RET activity) phenotype coexists with MEN 2 (gain of activity) in the same individual. None of the families reported thus far has a codon 634 or tk domain mutation; almost all have mutations in codons 618 or 620, and a few have mutations in codon 609. Some families with HSCR alone and no MEN 2 features have also been reported to have codon 609 mutation (68). The specificity of mutation in these families suggests the explanation. These mutations are weakly activating, but their signal cannot be augmented by ligand (52). The weak activity at the wrong time in development is sufficient to initiate C-cell and adrenal tumor formation, but without augmentation by ligand-induced signaling, it is insufficient on some genetic backgrounds to rescue the developing enteric neuroblasts from apoptotic death, and hence the HSCR phenotype results. The occurrence of the dual MEN 2/HSCR phenotype in only a few families and an impression that nuclear families are affected within the large pedigrees that have been reported are consistent with an explanation that involves genetic background as a key determinant.

Several other features of MEN 2 phenotypes remain to be explained in molecular terms. The absence of parathyroid involvement in MEN 2B might be because the presence of ligand is critical, and it is absent from parathyroid; however, this has yet to be demonstrated. Alternatively, the parathyroid cells may not express the signaling pathways...
that are critical in MEN 2B. The overwhelming predominance of MEN 2B-type mutations and other tk domain mutations rather than the cysteine mutations as somatic mutations in sporadic C-cell tumors also requires explanation (69–72). In sporadic pheochromocytomas, somatic ret mutations of any type are uncommon (73–75), and although MEN 2B-type mutations still outnumber cysteine mutations, the difference in frequency is nowhere near as marked. Each of these observations can be fitted into a scheme based on quantitative effects, depending on the presence or absence of ligand at different times in the relevant tissue, but the evidence to support such schemes is still lacking. An alternative explanation could be based on substrate specificity, but this would imply that the FMTC-associated tk domain mutations as somatic mutations in sporadic C-cell tumors have altered specificity, the relevant tissue, but the evidence to support such schemes is still lacking. A preliminary result suggests that interactions between variants of both DNA variants that may affect RET signaling, and some interesting but preliminary results suggest that interactions between variants of both domains and RET may be important in determining the HSCR phenotype (76–78). No data are as yet available relating to MEN 2.

Summary and Conclusions

Activating mutations of ret are associated with a range of clinical phenotypes within the MEN 2 syndrome. Current data suggest that the effects of these mutations in causing tumorigenesis occur through activation of the receptor at inappropriate times during development. This results in a perturbation of the normal differentiation of the relevant lineages, the consequences of which are not yet known at the level of the cellular phenotype. Different mutants result in different levels of activation, which in some cases can be further enhanced by the effects of ligand. The level of activation may have a major effect in determining the spectrum of tissues that is involved. Variation in phenotypes within the same family suggests a role for genetic modifiers, which may also work through quantitative effects. Detailed data on the expression of RET and its ligands at different times in different tissues will help in framing these hypotheses, which can be tested by the construction of models in transgenic mice and subsequently by the analysis of candidate genes in human material.

Acknowledgments

I thank the members of my laboratory and many colleagues in the MEN 2 field for stimulating discussions and, in particular, Dr. Francesca Carlonigou for valuable suggestions. I apologize that many excellent papers have not been quoted due to space limitations.

References


Discussion

Dr. Samuel Wells: As you know, there’s been a description of cutaneous lichenoid amyloidosis by Bob Gagel and others; I think that they didn’t find any specific mutation associated with it at that time, and I wonder if you have had further information on that. I also wonder if, in the very large number of families that you have followed with genotype-phenotype correlation, you’ve found any evidence of a mutation associated with disease progression, especially in MEN 2A, where some patients do have very aggressive tumors.

Dr. Ponder: Sam Wells (who has been in this field longer than I have) has asked two questions about clinical phenotypes. In some families, there is a skin condition, cutaneous lichenoid amyloidosis, in the interscapular area. The mechanism for this is not clear. It has been suggested that it is something to do with a malfunction in the sensory nerves of the dorsal root ganglion that leads to an abnormality in innervation of the skin and somehow to this phenotype. The particular question was whether it is associated with any one mutation. In the families that have been looked at, it has been associated pretty consistently with codon 634 mutations.

The other question was this: in the large set of families that we have been following, are there any correlations between particular mutations and the aggressiveness of disease or disease progression? It seems that in the families with FMTC, that is, with only MTC, the disease does seem to have a more indolent course on average. There are a couple of large families, which you were the first to report, in which hardly anybody had died of the disease. It’s hard to say whether that really holds up in smaller “MTC only” families. There is a generalization that the codon 609, 618, and 620 MEN mutants perhaps have a less aggressive clinical course, although I think we’ve all seen exceptions to that. There is no doubt that in MEN 2B families, the disease tends to present earlier. Whether MEN 2B disease is actually more aggressive, stage for stage, than MEN 2A after that point, I’m not so sure, and we have some data that we haven’t published suggesting that if you line everybody up at the same starting line with clinical presentation and then follow for survival, it may not be all that different in MEN 2B than in MEN 2A. Your clinical experience is greater than mine in this respect, so you might have a comment on that. Within the MEN 2A families, I don’t think I can say that I have seen a correlation with specific mutations.

Dr. Alfred Knudson: Can you comment on the current state of knowledge of the difference between C cells that are hyperplastic and those that are transformed? There was a thought at one time that there might be a tumor suppressor gene on chromosome 1, but I don’t know what your present thinking is on that.

Dr. Ponder: I am embarrassed to say that there has been little done on this for some years. About 5 years ago, Lois Mulligan in my laboratory did a very comprehensive LOH analysis of MTCs and found that in general these were chromosomally stable, but there were five regions in which there was a frequency of LOH above, I think, 10%, of which the most common was the short arm of chromosome 1. We didn’t have very good localizing information. We’ve always thought that it would probably be the same gene as the neuroblastoma gene, which was thought to be on chromosome 1p. But we don’t have any new data. We tried to do an analysis like Nick Dracopoli did years ago in melanoma, looking at the sort of sequence and the roll out of these LOHs in the evolution of these tumors, but we didn’t really come up with any good data suggesting a particular sequence of events. Information about oncogene activation in MTCs is very scanty. Studies have been made, but to my knowledge, no oncogenes have thus far been shown to be frequently activated. So the events at the genetic level in the progression of MTCs are still very obscure. To specifically address your question about the C cells, we don’t know whether those C-cell hyperplasias are the direct manifestation of RET or whether there’s a second somatic event involved already at that stage. In principle, by microdissecting the hyperplastic foci and studying them with comparative genomic hybridization and so forth, we ought to be able to find that out, but we just haven’t done that, and I don’t know that anybody else has.
The Phenotypes Associated with ret Mutations in the Multiple Endocrine Neoplasia Type 2 Syndrome

Bruce A. J. Ponder


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/59/7_Supplement/1736s

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cancerres.aacrjournals.org/content/59/7_Supplement/1736s.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.